Ruminal and intestinal digestibility of rumen protected lysine and its effect on production responses in Merino rams

by

Janien Uys

Thesis presented in partial fulfilment of the requirements for the degree
Master of Agricultural Science

at
Stellenbosch University
Department of Animal Science, Faculty of Agri Sciences

Supervisor: Dr. J.H.C. Van Zyl
Co-supervisor: Prof. C.W. Cruywagen
Co-supervisor: Dr. J.J.E. Cloete

March 2020

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2020

Abstract

Title	:	Ruminal and intestinal digestibility of rumen protected lysine and its effect on production responses in Merino rams.
Candidate	:	Janien Uys
Supervisor	:	Dr. J.H.C van Zyl
Co-Supervisor	:	Prof. C.W. Cruywagen and Dr. J.J.E Cloete
Institution	:	Department of Animal Sciences, Stellenbosch University
Degree	:	MSc Agric (Animal Science)

Wool is a large export commodity in South Africa where the price is determined by supply and demand. The average clean wool price per kilogram for the 2017/2018 wool clip season was R183.84 (top lines). This represents a price increase of approximately R30.00/kg (16.3%) in just one clip season. Therefore, improving the production of wool or optimizing the cost of the production of wool is of importance to the economic contribution of this farming sector.

A major objective in any animal production system is to increase the efficiency of converting nutrients into animal products. Some research suggested that the fibre growth of sheep may be limited by protein content that could be inadequate in the diet. These studies also reported that additional protein supplementation can influence wool yield. However, it was also reported that dietary protein supplementation had a negative influence by increasing fibre diameter. Another study reported that when ruminal degradation of high quality protein is reduced, substantial increases in the growth rate of wool is possible. Microbial protein (MP) alone is likely to meet the maintenance requirements of an animal but it is often insufficient to meet the demand for optimal production. Currently, various attempts have been made to develop and produce amino acids (AA) that escape degradation in the rumen. Due of the large quantities of AA that are degraded in the rumen, there is a lack of information on the availability of AA in the lower digestive tract of ruminants. This has prompted researchers to develop concepts to protect proteins from ruminal degradation, increase the supply of proteins for production and reduce nitrogen losses as urea via urine. Such methods include structural manipulation to produce AA analogues and AA coated with resistant materials. The capsule can thus resist degradation of AA in the neutral pH-environment of the rumen, but the AA would be made available for enzymatic digestion at the lower pH of the small intestine.

Little research has been done, not only on the use of synthetic AA in ruminants, but more so on wool producing sheep. The purpose of the current study was thus to determine whether or not rumen-protected lysine could improve the wool production of Merino sheep. More so wool growth, thus fibre length, fibre diameter, staple strength, fibre curvature and clean fleece weight, by supplementing the diet with high or low levels of bypass lysine or rumen degradable lysine.

The objectives of the study were to investigate the effect of different levels and type of dietary lysine on the production parameters of Merino rams. A secondary objective was thus to determine the difference in rumen degradability and intestinal digestion of rumen degradable lysine and bypass lysine. The final objective was to determine the absorption of this dietary lysine in the lower digestive tract when considering different inclusion levels of degradable and bypass lysine.

In the first trial, 40 Merino rams were supplemented with a diet that contained either high or low levels of bypass lysine and rumen degraded lysine. Merino rams with an average weight of 37kg and 1.5 years old, were fed for three months and during this period the feed intake were recorded daily to calculate the average daily intake (ADI) and the sheep were weighed weekly to calculate average daily gain (ADG). Blood samples were taken at the beginning of the trial and once during the trial to determine lysine absorption into the blood plasma. At the end of the trial, all the rams were sheared to determine wool production parameters throughout the trial. This study showed that supplementation of the bypass AA significantly ($P \leq 0.05$) increased the dry matter intake (DMI) of the rams. None of the measured wool parameters showed any differences between treatments. The blood plasma lysine levels were only significantly higher ($P \leq 0.05$) for the diets high in lysine but were not affected by degradability type. It was concluded that the use of rumen protected lysine as a supplementation in Merino rams showed little response to production parameters.

The second part of this study was to determine the apparent ruminal and intestinal digestibility values of a commercial lysine product by means of a three-step in vitro procedure, commonly referred to as the Ross Assay. The trial was done as a randomized block design with two types of commercial lysine products (rumen degradable and rumen protected) and six replications, where three cows were used as rumen fluid donors in two separate runs. The modified Ross Assay used in the trial was divided into two phases. This first phase represented the ruminal degradation phase and the second phase represented intestinal digestion. No significant differences in ruminal degradation or intestinal digestibility were observed between the two products. It was thus concluded that the encapsulation of the bypass lysine product did not decrease *in vitro* rumen degradation and neither did it increase the availability of lysine for absorption in the lower digestive tract.

Uittreksel

Titel	:	Ruminale- en intestinale vertering van rumenbeskermde lisien en die invloed daarvan op produksieparameters van Merinoramme.
Kandidaat	:	Janien Uys
Studieleier	:	Dr. J.H.C van Zyl
Mede-studieleiers	:	Prof. C.W. Cruywagen en Dr. J.J.E Cloete
Instansie	:	Departement van Veekundige Wetenskappe, Universiteit van Stellenbosch
Degree	:	MScAgric (Veekunde)

Wol is grootliks ‘n uitvoerkommoditeit in Suid-Afrika waar die prys deur vraag en aanbod bepaal word. Die gemiddelde skoonwolprys per kilogram vir die 2017/2018 wolskeerseisoen was R183.84 vir die toplyne. Hierdie prys verteenwoordig ‘n toename van ongeveer R30.00/kg (16.3%) in net een skeerseisoen. Die optimering van wolproduksie, sowel as die koste van wolproduksie, is van kardinale belang tot die ekonomiese bydrae van hierdie boerderyvertakking.

Die hoofdoelwit in enige veeproduksiesisteem is om die doeltreffendheid van omskakeling van nutriënte na diereprodukte te verhoog. Navorsingsresultate dui aan dat die veselgroei van wolskape beperk kan word deur onvoldoende dieëtproteïeninname. Hierdie studies het ook aangedui dat addisionele dieetproteïen wolopbrengs verhoog, maar dat dit terselfdertyd die veseldeursnit benadeel deur dit te verdik. Verdere navorsing het aangedui dat ‘n verhoging in die groeitempo van wol moontlik is indien proteïendegradering in die rumen verlaag kan word. Mikrobiese proteïenproduksie alleen voldoen normaalweg aan die onderhoudsvereistes van die dier, maar dit is dikwels onvoldoende om aan hoogproduseerende diere se vereistes te voldoen. Daar word tans verskeie pogings aangewend om aminosure te vervaardig wat degradering in die rumen vryspring. As gevolg van die groot hoeveelheid aminosure wat deur mikrobes in die rumen verteer word, is daar ongelukkig nog nie veel kennis oor die beskikbaarheid van aminosure in die laer spysverteringskanaal van herkouers nie. Dit veroorsaak dat navorsers verskeie konsepte ontwikkel wat ten doel het om dieetproteïen te beskerm teen rumendegradering. Hierdie konsepte het ten doel om proteïen vir produksie te verhoog, asook om stikstofverliese in die vorm van ureum via uriene te verlaag. Hierdie metodes sluit in stukturele manipulasie van aminosuur-analoë, asook om aminosure te kapsuleer met weerstandige materiale. Hierdie kapsulerings poog om die afbraak van aminosure deur rumenmikrobe in die rumenomgewing te weerstaan, maar om dit dan weer by die laer pH van die dunderm beskikbaar te stel vir ensiematiese vertering.

Weinig navorsing is tot dusver op die gebruik van sintetiese aminosure in herkouervoeding gedoen en nog minder in wolproduserende skape. Die doel van hierdie studie was dus om te bepaal of die voeding van rumenbeskermde lisienprodukte die wolproduksie van Merinoskape kan beïnvloed. Wolproduksie sluit vesesllengte, -deursnit, -kartel, staplesterkte, en skoon opbrengs in. Wolproduksieparamenters van Merinoramme is bepaal na die voeding van verskillende kombinasies van hoë- en lae vlakke van beide degradeerbare en nie-degradeerbare lisienprodukte. Die doelwit van hieridie studie was eerstens om die invloed van verskillende vlakke van dieetlisien op verskeie produksieparameters van Merinoramme te ondersoek. ‘n Tweede doelwit was om te bepaal of daar ‘n verskil tussen die produkte is in terme van rumendegradeerbaarheid en vertering in die laer spysverteringskanaal Die finale doelwit was om te bepaal of daar ‘n verskil in die absorpsie van lisien in die laer spysvereringskanaal van Merinoramme is indien verskillende insluitingsvlakke van rumendegradeerbare- en verbyvloeilisien gevoer word.

In die eerste eksperiment is 40 Merinoramme se diëte met verkillende aanvullings van hoë- of lae vlakke van verbyvloeilisien en rumendegradeerbare lisien aangevul. Agtien maand oue Merinoramme met ‘n gemiddelde gewig van 37kg het die behandelings vir drie maande ontvang waartydens die daaglikse voerinnames bepaal is ten einde die gemiddelde daaglikse inname te bereken. Die skape is eenmaal per week geweeg om die gemiddelde daaglikse toename te bereken. Bloedmonsters is versamel om die plasmalisienvlakke voor en gedurende die proef te bepaal. Aan die einde van die proef is al die ramme geskeer om wolproduksieparameters na afloop van die proef te bepaal. Hierdie studie het aangedui dat dieetaanvulling van verbyvloeilisien die droëmateriaalinname van die ramme betekenisvol ($P \leq 0.05$) verhoog het. Geen verskille in enige van die wolparameters is tussen behandelings waargeneem nie. Die plasmalisienvlakke was slegs betekenisvol hoër ($P \leq 0.05$) vir die diëte hoog in lisien, maar is nie beïnvloed deur lisiendegradeerbaarheid nie. Die gebruik van rumenbeskermde lisien as ‘n aanvulling vir Merinoramme het produksieparameters weinig beïnvloed.

Die tweede deel van hierdie studie het gepoog om die verdwyning van ‘n kommersiële lisienproduk in die rumen, sowel as die laer spysverteringskanaal, te bepaal deur middel van die Ross *in vitro* metode. Die proef is as ‘n ewekansige blokontwerp gedoen met twee tipes kommersiële lisienprodukte (rumen degradeerbare- en verbyvloeilisien) en ses herhalings waar drie koeie in twee verskillende sessies gebruik is. Die gemodifiseerde Ross-metode wat vir hierdie studie gebruik was, is in twee fases verdeel. Die eerste fase het ruminale degradering gesimuleer en die tweede fase het intestinale vertering gesimuleer. Die resultate van hierdie studie het geen betekenisvolle verskille tussen die degradeerbare en nie-degradeerbare lisienprodukte getoon nie. Die studie het verder aangedui dat daar geen verskille tussen die produkte was ten opsigte van die intestinale vertering nie. Die gevoltrekking is gemaak dat die kapsulering van die verbyvloei lisienproduk nie die rumendegradeerbaarheid daarvan kon

verlaag nie en gevolglik ook nie intestinale lisienbeskikbaarheid in die laer SVK kon verhoog nie.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- Dr. Brink van Zyl, for completely guiding me in this topic, sharing his knowledge and motivation.
- Prof. Chrisjan Cruywagen, for sharing his vast knowledge on this subject over various cups of coffee.
- Dr. Jasper Cloete, who made valuable contributions.
- Gert Cronje, Alex Sly and Juan-Louis Fourie, for all the advice regarding the product, the Ross Assay and the formulation of the feeds.
- Afgri Animal Feeds, for supplying the feed for the *in vivo* study, a sample of the product and for analyses done.
- The Western Cape Department of Agriculture, for the use of their facilities and animals for the *in vivo* trial.
- Steyntjie, Davey, Abraham and Clinton, for the assistance in feeding and weighing the sheep throughout the trial.
- Beverley Ellis, Micheal Mlambo and Janine Booyse for the assistance in the laboratories.
- Brahm Burger, for struggling with me through all the obstacles and finding solutions for all of them.
- Cape Wools, for the financial assistance.
- Stellenbosch University Support Bursary, for the postgraduate bursary.
- My parents, Eduan and family, for the continuous interest and encouragement.

Note

This thesis is presented as a compilation of 5 chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Animal Science.

List of Figures

List of Tables

List of Abbreviations

AA:	Amino Acids
ADF:	Acid Detergent Fiber
ADG:	Average Daily Gain
ADI:	Average Daily Intake
CV:	Coefficient of Variation
CF:	Crude Fiber
CFW:	Clean Fleece Weight
CP:	Crude Protein
CSND:	Corn Silage Neutral Detergent Residue
DM:	Dry Matter
DMI:	Dry Matter Intake
EE:	Ether Extract
FC:	Fiber Curvature
FD:	Fiber Diameter
HLP:	High Levels Lysine Protected
HLU:	High Levels Lysine Unprotected
ID:	Intestinal Digestion
IWTO:	International Wool Textile Organization
LDT:	Lower Digestive Tract
LLP:	Low Levels Lysine Protected
LLU:	Low Levels Lysine Unprotected
MP:	Microbial Protein
MRT:	Mean Retention Time
NDF:	Neutral Detergent Fiber
OM:	Organic Matter
RD:	Ruminal degradation
SDFD:	Standard Deviation of Fiber Diameter

SG:	Specific Gravity
SL:	Staple Length
SS:	Staple Strength
TMR:	Total Mixed Ration
UPLC:	Ultra Performance Liquid Chromatography
WTB:	Wool Testing Bureau of South Africa

Table of Contents

Chapter 1:
General introduction

1.1 History

One of the oldest agricultural industries in South Africa is the wool industry. The first sheep arrived in the Cape in 1789, after which the wool industry was soon established (Merino SA, 2019). Currently Merino sheep are found in almost every district of South Africa. Merino sheep are spread from the drier Northern Cape Province, the fertile lands of the winter rainfall areas of the Western Cape to millions of Merino sheep in the Karoo veld and grassland of the Eastern Cape and Free State of Southern Africa. Well-known Merino breeders with large, top quality, flocks are also found in East Griqualand of Kwazulu-Natal and the most parts of Mpumalanga of South Africa (Merino SA, 2019).

1.2 Current market

As wool is produced on small holder and commercial farm scale in South Africa, the industry provide employment and livelihood to a vast amount of South Africans (Kuffner and Popescu, 2012). Wool is largely an export commodity in South Africa where earnings are determined by foreign exchange rate. This income plays an important role in the annual agricultural foreign currency income of South Africa and contributed over 5 billion rand in 2018 (Merino SA, 2019).

Free market wool price is determined by supply and demand (Dlodlo *et al.,* 2009). The 2017/2018 wool clip season yielded an average of R183.84 (top lines) per kilogram. This was approximately R30.00/kg higher than the previous season (Cape Wools SA, 2019).

Due to the dynamic versatility of wool, it is used in numerous sectors ranging from fashion, flooring, architecture and even medical industries (Kuffner and Popescu, 2012). This is a clear indication of rapid economic growth seen in the past years and is indicated in Figure 1.1 (Cape Wools SA, 2018). Therefore, improving the production of wool or optimizing the cost of the production of wool is of importance to the economic growth of this farming sector.

Figure 1.2 shows that the total wool mass sold per year in South Africa was fairly constant over the last 10 years (Cape Wools SA, 2018). Wool mass produced by the Merino breed exceeded more than half of the total amount of wool produced in South Africa (Figure 1.2). The modern Merino breed is economically considered the ideal natural wool producing breed as it produces minimal waste in addition to high quality meat (Kuffner and Popescu, 2012).

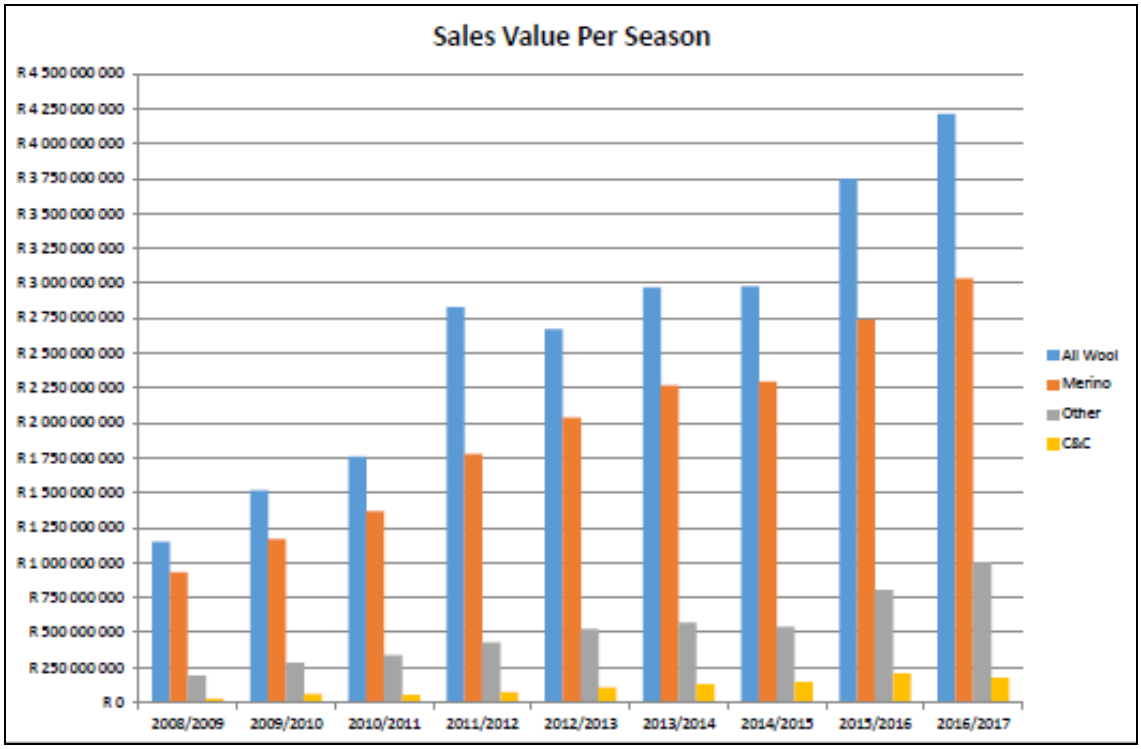

Figure 1.1 The total wool sales value per season in South Africa (Cape Wools SA, 2018).

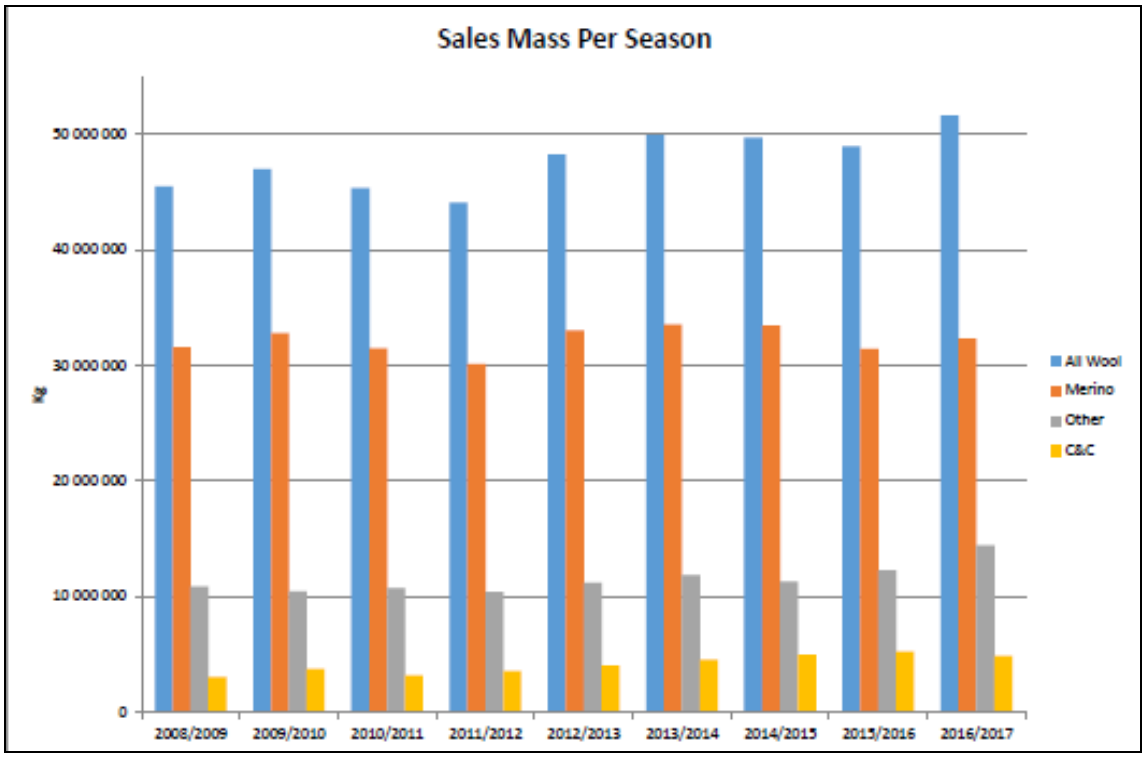

Figure 1.2 The total wool sales mass per season in South Africa (Cape Wools SA, 2018).

1.3 History of amino acids in animal feed

Before amino acids (AA) as a supplement in animal feed became of interest, casein was used as supplement for grazing sheep (Meissner and Todtenhofer, 1998). Through further studies an increase in N-retention was seen when casein was supplemented in the diet (Meissner and Todtenhofer, 1998). Reis *et al.* (1992) indicated that the casein supplementation lead to a large increase of essential AA concentration in the lower digestive tract (LDT). These authors then suggested that the higher essential AA concentration found in the casein was absorbed in the tissues of the animals (Reis *et al.,* 1992).

Results of these studies (Reis *et al.,* 1992) stimulated further research on specific AA and by-pass AA supplementation in animal diets. A large component of this research was focused in the dairy industry. For example studies done by Socha *et al.* (2005) to improve the intestinal AA supply of dairy cows, Awawdeh (2016) who researched the effect of rumen protected AA on the milk production and plasma AA of dairy cows and Patton, (2010) who studied the effect of rumen protected AA on the intake, milk production and true milk protein concentration in dairy cows.

1.4 The importance of amino acids in the diets of ruminants

Amino acids is the building blocks of all proteins required for optimal growth, production and maintenance in all animals (Kung and Rode, 1996). When fed to ruminants, like sheep, it is difficult to predict the quality and quantity of the AA that will be absorbed by the animal. This is due to the microbial presence in the rumen of these animals where the AA is first subject to microbial degradation (Kung and Rode, 1996). Microbial protein (MP) is likely to only meet the maintenance requirements of an animal (Kamalak *et al.,* 2005), however to achieve optimal production level of the animal MP is insufficient. Therefor the need to formulate feeds with specific AA profiles was identified and has now led to new studies looking into the development of rumen protected AA.

1.5 New advances in rumen protected amino acids

Large quantities of AA are microbially degraded in the rumen (Ohsumi *et al.*, 1994). This has led researchers to develop concepts to protect AA from microbial degradation. Thus increasing the supply of these AA for production and reducing nitrogen losses as urea in the urine (Annison, 1981).

Various methods have been investigated to create rumen by-pass AA and include the following:

- Heat treatment of AA, which cause it to be more resistant to the enzymatic hydrolysis of rumen microbes (Belits *et al.*, 2005).
- Formaldehyde treatment was explained by Czerkawski (1986). This treatment process created cross-links between the amino groups of protein forms and thus reduce the susceptibility of microbial degradation in the rumen.
- Yeast cells was found to use the vacuoles to that accumulate AA and further transport AA to the LDT (Dawson *et al.*, 1990).
- Encapsulation is a method that consist of structural manipulation to produce AA analogues and AA coated with resistant materials (Kamalak *et al.*, 2005).

1.6 Lysine use as feed supplement for ruminants

Lysine is one of the most limited AA commonly used as a raw material for animal feed formulation (Robinson *et al.*, 1998) and synthetic lysine is widely used in monogastric feed formulation as a supplement (Toledo *et al.*, 2014).

The use of encapsulated AA, especially methionine and lysine, has been evaluated in the dairy industry due to these AA being the first limiting AA in lactating dairy cows (Robinson *et al.*, 1998). Despite variable responses in the milk yield, slight positive increases in milk protein production were observed (Kincaid and Cronrath, 1993).

Research done by Nolte and Ferreira (2005) on sheep established the effect of rumen degradable protein level and source on the essential AA in the lower digestive tract (LGT).

1.7 Objectives

A study was conducted at the University of Stellenbosch, Department of Animal Science and Department of Agriculture, Western Cape, Elsenburg Research Farm to determine the influence of a commercial protected lysine on the wool production parameters of Merino rams. The objectives of the study were to determine:

- The effect of level of dietary lysine on wool production parameters of Merino rams
- Evaluate the effect of lysine type (degradable or by-pass) on the production parameters of Merino rams
- Evaluate the absorption of dietary lysine in the LDT between four different treatment diets with a combination of either high or low inclusion level of either degradable or by-pass lysine.
- The difference in rumen degradability and intestinal digestibility of rumen degradable lysine and by-pass lysine

1.8 References

Annison, E.F., 1981. The role of protein which escapes ruminal degradation. (Recent Advantages in Animal Nutrition in Australia) (Unit; D. J. Farrell, Ed.).

Awawdeh, M.S., 2016. Rumen-protected methionine and lysine: effects on milk production and plasma amino acids of dairy cows with reference to metabolizable protein status. Journal of Dairy Research, 83, 151–155.

Belits, H.D., Grosch, W. and Schieberle, P., 2005. Reaction involved in food chemistry. In: Food Chemistry. (3rd ed.; Springer Berlin, Ed.).

Cape wools SA, 2019. *Wool production reports*. Retrieved April 13, 2019 www.capewools.co.za

Czerkawski, J.W., 1986. An introduction to rumen studies (1st Ed.).

Dlodlo, N., Botha, A.F. and Metelerkamp, R.G.M., 2009. A distributed knowledge-based system for the optimum utilization of South African wool. Suid-Afrikaanse Tydskrif Vir Natuurwetenskap En Tegnologie, 28(3), 187–204.

Kamalak, A., Canbolat, O., Gurbuz, Y. and Ozay, O., 2005. Protected Protein and Amino Acids in Ruminant Nutrition. Journal of Science and Engineering, 8(2), 84–88.

Kincaid, R.L. and Cronrath, J.D., 1993. Effects of added dietary fat and amino acids on performance of lactating cows. Journal of Dairy Science, 76(6), 1601–1606.

Kuffner, H. and Popescu, C., 2012. Wool fibers - 8 (1st ed.; R. M. Koslowski, Ed.).

Kung Jr. L. and Rode L.M., 1996. Amino acid metabolism in ruminants. Animal Feed Science Technology, 59, 167–172.

Meissner, H.H. and Todtenhofer, U., 1998. Influence of casein and glucose or starch supplementation in the rumen or abomasum on utilization of hay by sheep. S. Afr. J. Anim. Sci, 19(1), 43–49.

Merino SA, 2019. *The history of the merino sheep*. Retrieved May 23, 2019, from www.merinosa.co.za

Nolte, J. v. E. and Ferreira, A.V., 2005. The effect of rumen degradable protein level and source on the duodenal essential amino acid profile of sheep. *South African Journal of Animal Science* 35, 12-56.

Ohsumi, T., Sato, H., Yoshihara, Y. and Ikeda, S., 1994. Selection and Breeding of Lysine-accumulating Saccharomyces cerevisiae as a Stable Source of Lysine in the Rumen. *Bioscience. Biotechnology. Biochemistry*, 58, 1302–1305.

Patton, R.A., 2010. Effect of rumen-protected methionine on feed intake, milk production, true milk protein concentration, and true milk protein yield, and the factors that influence these effects: A meta-analysis. *Journal of Dairy Science*, *93*, 2105–2118.

Reis, P.J., Funks, D.A. and Munro, S.G., 1992. Effects of Abomasal Protein and Energy Supply on Wool Growth in Merino Sheep. Aust. J. Agric. Res, 43, 1353–1366.

Robinson, P.H., Chalupa, W., Sniffen, C.J., Julien, W.E., Sato, H., Watanabe, K and Miner, W.H., 1998. Ruminally Protected Lysine or Lysine and Methionine for Lactating Dairy

Cows Fed a Ration Designed to Meet Requirements for Microbial and Post-ruminal Protein. *Journal of Dairy Science*, 81, 1364–1373.

Socha, M.T., Putnam, D.E., Garthwaite, B.D., Whitehouse, N.L., Kierstead, N.A., Schwab, C.G. and Robert, J.C., 2005. Improving intestinal amino acid Supply of pre-and postpartum dairy cows with rumen-protected methionine and lysine. *Journal of Dairy Science*, *88*, 1113–1126.

Toledo, J.B., Furlan, A.C., Pozza, P.C., Carraro, J., Moresco, G., Ferreira, S.L. and Gallego, A.G., 2014. Reduction of the crude protein content of diets supplemented with essential amino acids for piglets weighing 15 to 30 kilograms. R. Bras. Zootec, 43(6), 301–309.

Chapter 2:
Literature review

2.1 Wool

Wool fibre is a natural, sustainable and biodegradable material. All of these properties are highly valuable and desired in the textile industry (Kuffner and Popescu, 2012). Natural wool fibre grown by sheep further offers practical characteristics that exceed man-made fibres (Kuffner and Popescu, 2012).

2.1.1 Wool composition

When considering the elemental analyses of wool as done by Popescu and Höcker (2007), comparable percentages can be seen when comparing elements in wool from various wool types: carbon around 50%, hydrogen at 7%, oxygen at 22%, nitrogen at 16% and sulphur at 5%. The high sulphur content seen in wool is the result of the high amount of sulphur-containing amino acids (AA) present, for example, methionine and cysteine. The high water and nitrogen content measured in wool allows the fibre to be flame retardant without any other chemical treatments (Kuffner and Popescu, 2012). Wool is therefore classified as a polymer consisting of AA (Kuffner and Popescu, 2012). Therefore, most previous research done on nutritional factors that affect wool growth focused on sulphur-containing amino acids (Ramada *et al.,* 2017).

2.1.2 Wool growth rate

The physiological growth of fibre such as wool is defined as being dependent on the constant division of cells in the wool follicle (Williams and Winston, 1987). This wool growth rate varies due to the genotype, physiological factors and environmental factors (Khan *et al*., 2012). Distinct differences between sheep breeds in the ability to grow wool have been reported. Merino sheep have a greater follicle density than other woolled breeds and thus they grow more wool (Williams, 2000).

Various parameters of wool contribute to wool yield in sheep. Staple length (SL), staple strength (SS), clean fleece weight (CFW), fibre diameter (FD) and fibre curvature (FC) all contribute the yield (Safari *et al.*, 2005). When sheep are selected for an increase in wool growth it was found that the association was on the microscopic level of the skin. Important aspects are the proportion of primary to secondary follicles, follicle density, the skin depth of the follicle, rate of integration of cortical cells into the fibre and mitotically active follicles (Williams and Winston, 1987; Nancarrow *et al.,*1998). Williams (2000) also found that Merino sheep have

wool follicles that are set deeper in the skin and that they usually have more follicles per skin surface area, enabling them to grow more wool than other woolled breeds.

2.1.3 Fibre structure

Wool fibre is made up of two parts, namely the follicle and the shaft. The follicle is located below the surface of the skin and produces the fibre material. The shaft grows from the follicle and is predominately composed of the fibrous protein α-keratin (Kuffner and Popescu, 2012). One of the best representatives of the α-keratin fibre is wool (Kuffner and Popescu, 2012). Merino wool fibre comprises of two types of cells, namely the ortho and para-cortex, that assemble as two strands that coil together and causes (Figure 2.1) the crimp seen in merino wool fibres (Kuffner and Popescu, 2012).

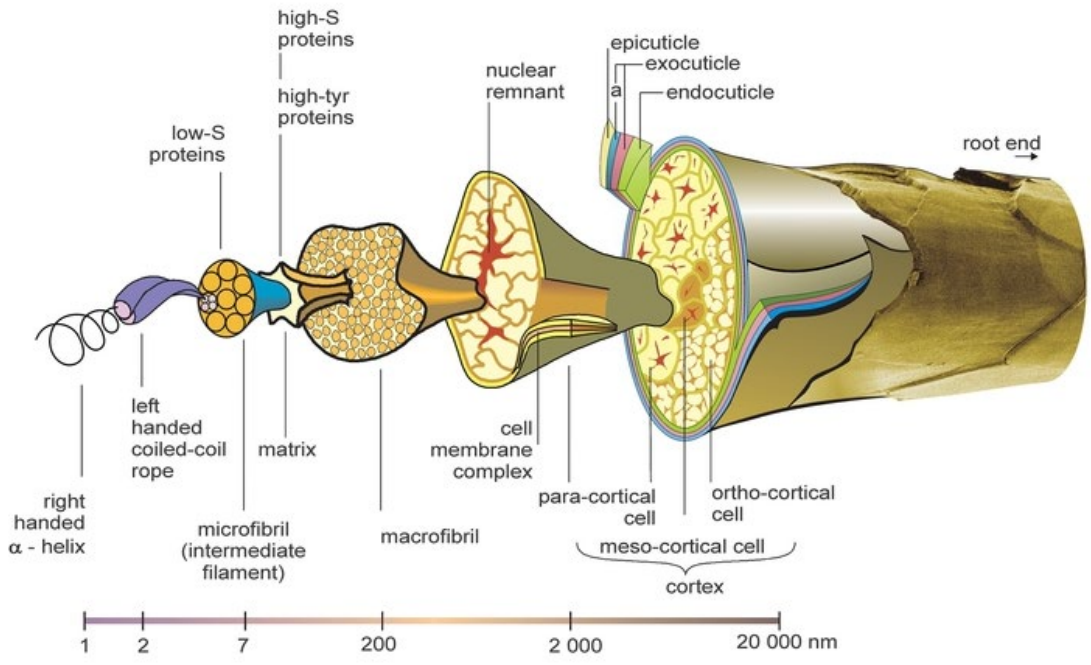

Figure 2.1 The structure of a merino wool fibre (Marshall *et al.*, 1991).

2.1.4 Fibre Length

The length of the fibre is predominantly referred to as the SL of the fleece. The SL can be explained as a function of individual fibre lengths and the degree of crimping (Khan *et al.*, 2012). Wool SL is proved to be phenotypically directly correlated to the live weight of the sheep (Huisman and Brown, 2008). However, the results of Mcgregor *et al*. (2016) contradicted that of Huisman and Brown (2008) showing that SL tends to decrease with age as FD increase. It can, therefore, be derived that sheep live weight will only directly affect SL until a certain age. Therefore, it is concluded that wool growth efficiency is not only a function of a ratio of fibre length and diameter (Mcgregor *et al.,* 2016).

2.1.5 Follicle density

The rate of fibre production is around 1 cm per month for a single follicle on the skin (Kuffner and Popescu, 2012). However, the number of follicles per skin area, also known as the follicle density, differs between sheep. Follicle density thus regulates the amount of wool produced per sheep as a fleece with a higher follicle density may lead to a higher amount of fleece produced (Kuffner and Popescu, 2012). In comparison research done by Mcgregor *et al.* (2016) showed that sheep with a larger frame size or live weight may have a larger skin surface area and thus less follicle density, leading to fibres with larger diameters and thus resulting in a higher fibre mass and thus fleece mass.

2.1.6 Fibre diameter

The diameter of wool fibre varies between different breeds of sheep as well as between sheep in the same breed and even the same flock because FD is largely determined by underlying genetic factors (Figure 2.2). Environmental and managerial factors such as weather and feed may also affect FD to a lesser extent (Kuffner and Popescu, 2012). The most common range of FD seen in Merino sheep of South Africa varies from 17-24 micron and is classed in three different groups: fine, medium and strong (Friend and Robards, 2006). The FD has the greatest influence on the determination of the price of raw wool (Matebesi, Van Wyk, and Cloete, 2009). In general, finer wool with a lower FD will fetch a higher price (Friend and Robards, 2006). An increase in FD, however, leads to an increase in clean fleece weight (Williams, 2000).

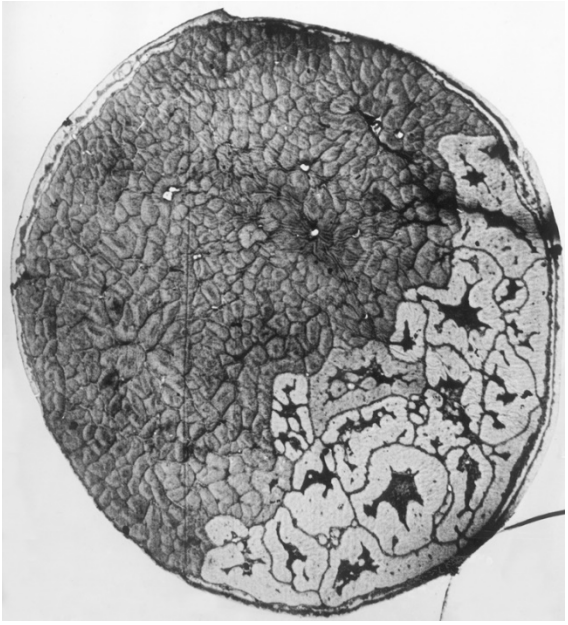

Figure 2.2 A cross-section of a Merino wool fibre (Marshall *et al.*, 1991).

2.1.7 Staple strength

Fibre diameter and the deviation in FD of the wool staple due to a variety of environmental factors that affect the live weight of the animal, as previously explained in section 2.1.6, can also affect the strength of the wool fibre. These variations along the length of the staple may cause areas with lower FD that break more easily when extended during processing (Khan *et al.*, 2012). The phenomenon when wool breaks more easily is known as 'tender wool'. The strength of the wool fibre is determined by using objective methods where the soundness of the wool is tested by extending wool until it breaks (Collins and Chaikin, 1968; Friend and Robards, 2006). Rather than measuring the strength of one fibre the strength of a whole staple is determined. Research showed that the main factor affecting staple strength (SS) is the fibre diameter (Khan *et al.*, 2012). However, the SS is a multifaceted interaction between many factors, such as live weight changes, nutrition, physiological status, disease status as well as genetics (Mcgregor *et al.*, 2016). This multifaceted interaction often creates problematic management challenges on a farm. Friend and Robards (2006) observed that by controlling the nutrition of the woollen sheep in pens, one of the main factors influenced was the FD. These researchers also found that sheep that maintained their live weight better produced more stable wool (> 35N/ktex).

2.1.8 Fibre Curvature

The curvature seen in Merino wool is caused by the association of the two cell types in the skin that coil together and cause the fibre to crimp (Khan *et al.,* 2012). Campbell *et al.* (2011) postulated that the crimp in the wool is a function of time rather than SL after observing that an

increase in SL leads to a decrease in fibre crimping. A condition known as “doggy wool” is a condition in sheep where the crimp is severely lost (Khan *et al.,* 2012). The curvature of the fibre significantly affects the value of the wool as the curvature influence the processing of the final fabric (Hynd *et al.*, 2009).

2.1.9 Wool Colour

Unprocessed raw Merino wool has a variety of colours ranging from cream to yellow (Wang *et al.,* 2011). Wool colour alongside the other characteristics of wool is one of the determinants for the price of a certain batch of wool (Friend and Robards, 2006). It is known that wool with a yellow colour can attract significant price decreases in the wool markets due to the constricted range of colours to dye such wool (Dyer *et al.*, 2007).

Published results show significant heritability of wool colour, ranging from 0.39 to 0.56 (Benavides and Maher, 2003). The exact cause of the yellowing fleece has not yet been fully determined (Benavides and Maher, 2003). Therefore sheep breeders currently incorporate sheep with a clean wool colour as a genetic selection into their breeding plans (Cottle and Cowie, 2016). It was also found that wool colour is correlated with FD and selection of one trait may lead to the inheritance of the other. (Wang *et al.*, 2011).

Management practices also influence the colour of the wool. Benavides and Maher (2003) showed that wool sheered in the winter had a lower yellow fleece percentage than wool sheered in the summer.

2.2 Management practices affecting wool parameters

2.2.1 Age

Age is one of the primary factors affecting wool parameters. Khan *et al*. (2012) have stated that young sheep produce less wool per unit of feed intake due to the nutrient requirements for growth in these sheep. Maximum fleece weight was found between the ages of 3 to 5 years (Corbett, 2001). An increase in age in the Merino breed furthermore may lead to increases in FD and/or decreases in SL (Mcgregor *et al.*, 2016). A decrease in wool growth in older sheep may be due to the change in feed intake and preferences (Khan *et al.*, 2012).

2.2.2 Reproduction of ewe

Body condition maintenance of the ewe is critically important to ensure reproductive success in any well-executed management/breeding strategy. Thus the live weight management strategies will, therefore, prevent economic loses later in the reproductive cycle. Black and Reis (1979)

and Morley (1982) showed that seasonal variation in sheep (like pregnancy and lactation) may affect the wool parameters like fibre diameter. However, if the body condition is maintained throughout these seasonal changes wool growth will not be affected (Williams and Butt, 1989).

2.2.2.1 Pregnancy and lactation

Nutrient partitioning during pregnancy and lactation is the main cause of a decrease in quality as well as the quantity of wool. This phenomenon can be attributed to nutrients that will be directly used for foetal growth and to a lesser degree wool growth (Mcgregor *et al.*, 2016). Thus, the amount of dry matter intake in the sheep will increase during this period (SCA, 1990). The efficiency of dry matter intake to wool production will, however, decrease (SCA, 1990). As previously mentioned, pregnancy and lactation can be seen as seasonal changes affecting the live weight of the sheep and thus decreasing fibre diameter. However, research by Waters *et al.* (2000) indicated that these negative effects on wool growth and FD are only influenced by the current reproduction and not previous reproduction cycles. This can be explained by the compensatory weight gain positively influencing wool growth after lambs are weaned (Lee and Atkins, 1995).

Staple strength is another wool parameter that is commonly affected by pregnancy and lactation. Staple strength was found to decrease during pregnancy and lactation and even more so when the litter size of the ewe increased (Robertson *et al.*, 1996). This can also be attributed to the higher foetal demand for nutrients and a lesser extent to wool growth and wool soundness.

2.2.2.2 Pre- and post-natal development

Both primary and secondary follicle development commence during the pre-natal phase of development (Everitt and Taplin, 1967). Therefore, nutrition of the pregnant ewe is very important to ensure acceptable wool production of the progeny and might have an influence on both the long- and short-term wool production. Research done by Thompson *et al*. (2011) defined several responses to the influence of ewe weight on wool parameters of their progeny. In the first scenario, the live weight of the ewes throughout the gestation period was kept stable and the response showed that the progeny had finer wool (-0.2 µm/10 kg ewe live weight). However, in a second scenario, where the live weight of the ewes varied, the ewes that had an increase in live weight during the gestation period produced progeny with finer wool. This study showed that single born lambs tended to produce finer wool in comparison to twin lambs (Thompson *et al*., 2011). It can, therefore, be concluded that constant quality nutrition of the ewe, as well as the lamb, have the biggest influence on the economic value of the wool (Mcgregor *et al.*, 2016).

Although the live weight of the ewe was seen to have a significant influence on the wool parameters of the progeny, other factors like the gender of the lambs, as well as the litter size, may also affect the wool parameters. The study by Thompson *et al.* (2011) furthermore showed that single-born lambs produced significantly ($P < 0.001$) more wool than the twin-born lambs. In this research, the male lambs produced, on average over a 27-month period, more wool than the female lambs, but the wool produced by the female lambs were finer. Both these factors may be the cause of male lambs having a faster growth rate over a 27 month period (Thompson *et al*., 2011).

2.2.3 Parasites

Any form of ill health caused by either parasites, viruses or bacteria usually influence all types of animals and their production. The influence is either on a qualitative or quantitative level. Wool production in Merino sheep is no exception to this. The most common health problems in Merino sheep that are associated with management are gastrointestinal parasites, fleece and foot rot, blowfly strike and lice (Mcgregor *et al.*, 2016). Gastrointestinal parasites largely influence the FD of wool due to the negative effect it has on the live weight of the animal (Thompsona and Callinan, 1981). Fleece rot is caused by prolonged periods of wet wool that leads to the population of bacteria (*Pseudomonas aeruginosa*) that discolour the wool and reduce the market value significantly (Norris *et al.*, 2008). Similarly, as in the case of gastro-intestinal parasites, footrot affects the live weight of sheep and thereby decreases wool parameters such as FD (Mcgregor *et al.*, 2016). Fleece rot is one of the factors predisposing sheep to blowfly strike. Elevated stress levels from blowfly strike result in increased levels of adrenocorticotropic hormone (ACTH) and cortisol and may lead to ‘tender wool’ that is lost when sheered (Pierzchala-Koziec *et al.*, 2018).

2.3 Environment

The environment where sheep are kept can play a substantial role in the value of the fleece produced. Stains in the fleece generally cause a price reduction. These stains can be caused by biological and non-biological factors in the environment. This can range from bacteria or parasites, dipping solutions, urine of the sheep and even vegetable contamination (Mcgregor *et al.*, 2016).

Severe sunlight and exposure to other weather can decrease the quality of the fleece produced by the sheep. Extreme changes in weather conditions were found to influence newly sheered sheep the most due to more direct exposure of the skin to the environment. Severe weather can also influence the feeding behaviour of sheep. For example, when the

temperatures are high sheep will reduce mobility to keep their body temperature down (Khan *et al.*, 2012).

2.4 Nutrition

2.4.1 Change in feed intake

Changing the diet of the animal can affect production. Thus, as the live weight of sheep increase or decrease either due to maturing or seasonal changes in the natural pasture, wool growth will be affected (Mcgregor *et al.,* 2016). This implies that it is impossible to distinguish between the effects of nutrition on wool growth without it influencing the growth of the sheep. Earlier research by Allden (1979), showed that wool parameters, e.g. FD, change due to an increase or decrease or the quality of the nutrients availability. Khan *et al*. (2012) also stated that a variation in nutrient supply to the wool follicle can have a significant effect on not only the rate of fibre production but also the different characteristics of the fleece. According to the latter author, the nutritional level will thus also affect the live weight of the sheep. Therefore, wool parameters, especially FD, are directly affected by the type of feed the animals received throughout seasonal changes, whether physiologically or environmentally (Safari *et al.*, 2005 and Mcgregor *et al.*, 2016). De Barbieri *et al.* (2015) concurs with these results and reported that the most important factor affecting any wool parameter is the nutrient level of intake of a sheep, due to the increase in nutrients available for the sheep to produce wool.

Mineral deficiency can also influence wool parameters negatively. Mineral deficiency is usually a result of changes in nutrient supply due to a change in either feed intake or density. The main minerals that affect wool growth are zinc and copper. A deficiency in zinc may lead to brittle wool or a loss of crimp (Khan *et al.*, 2012). In severe cases, it may even lead to termination of wool growth and shedding of the fleece. A deficiency in copper may lead to a lower wool growth rate due to reduced feed intake (Khan *et al.*, 2012).

2.4.2 Effect of different roughages

In farming systems around the world, most sheep are farmed extensively were the quantity and quality of forage differ in separate times of the year (Khan *et al.*, 2012). This can thus also form part of the seasonal environmental changes. Research, however, lacks the effect of different roughages on wool growth. This is mainly due to the extreme variability of pasture types affecting quantity and quality between geographical areas and seasons.

2.4.3 Different protein sources

A study done by Galbraith in 2000 suggested that the fibre growth of sheep may be limited by protein content that could be inadequate in the diet. This study also reported that additional protein supplementation may influence the wool yield, but that it had a negative effect on the wool price by increasing FD. An increase in FD is generally accepted to be commercially unwanted due to a reduction of the comfort factor (Galbraith, 2000). Changes in the fractional protein synthesis rate and the total protein synthesis in the skin are associated with increased wool growth (Li *et al.*, 2008). Allden (1979) further reported that when rumen degradation of proteins is reduced in sheep, substantial increases in the growth rate of the wool are possible.

2.4.3.1 Casein

Before the use of by-pass protein and later by-pass amino acids (AA) concepts, casein was used as supplementation for grazing sheep (Meissner and Todtenhofer, 1998). Casein was found to increase wool growth rate significantly, even when combined with a feed of moderate energy content. In a study to evaluate the effect of casein on wool growth, casein was infused in the abomasum (Reis, 2000). These casein infusions lead to a large increase in essential AA concentrations in the lower digestive tract (LDT) (Reis *et al.*, 1992). Meissner and Todtenhofer (1998) observed that N-retention increased when casein was supplemented in the diet of sheep, they suggested that more essential AA were absorbed into the tissue of the sheep.

2.4.3.2 Fishmeal

Fishmeal is known to be one of the most frequently used rumen undegradable protein or more commonly known as by-pass protein sources. Other by-pass protein sources similar to fishmeal include poultry by-product, carcass, blood and feather meal (Kung and Rode, 1996). Safari *et al.* (2005) indicated that protein sources with sulphur-containing AA (methionine and cysteine) increase wool growth the most. Fishmeal is a good source of these sulphur containing AA, especially methionine (Kung and Rode, 1996). In South Africa, an expensive component like fishmeal was used in the formulation of animal feed; however, currently, the un-renewability of the raw material is forcing the industry to find different alternatives.

2.4.3.3 Plant protein

The main plant protein source used in animal nutrition is soya bean meal (Zargorakis *et al.*, 2018). Despite soya bean meal, sunflower meal, cotton meal, canola meal, lupin seeds and

many more plant-based protein raw materials are commonly used in animal feed (Zargorakis *et al.*, 2018).

Canola meal is seen as one of the main protein source used in formulated animal feed, especially for dairy cows (Mulrooney *et al.*, 2009). The popularity of canola meal has increased over the past years due to the increased interest in canola oil and therefore increased production of canola crops (Zargorakis *et al.*, 2018).

Lupin seeds are another popular protein supplement in dairy diets (Froidmont and Bartiaux-Thill, 2004). The availability of lupin seeds in South Africa has also increased in the past few years and is due to the ecological advantages thereof in crop rotation systems (Zargorakis *et al.*, 2018).

In a study done by Zargorakis *et al.* (2018) on the effect of replacing soybean meal with alternative plant protein sources in sheep diets, the authors reported that the nutritional value of the diets did not change when soybean meal was replaced with canola meal. These researchers also found that the use of lupin seeds in sheep diets was most advantageous as it improved the DM and nutrient intake, nutrient digestibility, average daily gain and feed conversion efficiency. These authors further recommend using lupin seed as a plant protein source not only in maintenance diets but also in ruminant diets formulated for higher production.

2.4.4 Absorption of amino acids

Amino acids are the building blocks of all proteins required for optimal growth, production and maintenance in animals (Kung and Rode, 1996). When fed to ruminants, such as sheep, it is difficult to predict the quality and quantity of the AA that will be absorbed by the animal. This is due to the microbial population in the rumen where the AA is first subjected to microbial degradation (Kung and Rode, 1996). Amino acids that are absorbed in ruminants originate from microbial protein (MP) or from dietary AA that escaped rumen degradation (Kung and Rode, 1996). The production of AA from MP synthesis is controlled by the quantity of plant organic matter fermentation in the rumen (Kamalak *et al.,* 2005). Nonetheless, MP alone is likely to meet the maintenance requirements (Kamalak *et al.,* 2005), but it is insufficient to meet the optimal production demands of the animal (Kaufmann and Lupping, 1982). The quantity of AA that escapes degradation in the rumen is dependent on factors such as AA solubility and the rate of passage to the LDT (Kamalak *et al.*, 2005).

Genetic selection for increased wool growth was also found to influence the effectiveness of using AA available to the body for wool production as well as the retention of ingested protein in the wool of the sheep (De Barbieri *et al.*, 2015). The absorption of free AA for production traits like wool production pertains in the lower digestive tract (LDT) (Safari *et al.*, 2005).

2.4.4.1 Prevention of rumen degradation

Lysine is one of the most limited AA in commonly used raw materials used for animal feed formulation (Robinson *et al.*, 1998) and is widely used in monogastric feed formulation as a supplement (Toledo *et al.*, 2014). Unfortunately, there is not much knowledge of the nutritional availability of AA in the LDT of ruminants, because large quantities of AA are microbially degraded in the rumen (Ohsumi *et al.,* 1994). This has sparked research to develop concepts to protect proteins from rumen degradation, increasing the supply of proteins for production and reducing nitrogen losses as urea in the urine (Annison, 1981). Research showed that increasing the availability of AA in the LDT results in an increase in animal production, such as wool (Kung and Rode, 1996). Several other studies showed that a mixture of AA, casein and other proteins increases milk yield and/or milk protein in dairy cows if it is absorbed or infused post ruminally (Ohsumi *et al.*, 1994). Thus, by preventing the degradation of certain AA in the rumen the absorption of these AA can be increased in the LDT (Ohsumi *et al.*, 1994). Furthermore, Savary *et al.* (2001) found that increased intake of rumen-protected lysine is linearly correlated with the absorption of these AA in the LDT.

A variety of methods and techniques have been developed to increase the supply of AA to the small intestine (Kung and Rode, 1996). By decreasing the AA retention times in the rumen, the rumen degradation of these AA can be reduced. Factors influencing this is the level of food intake, the particle size of the diet, concentrate to roughage ratio and rate of rumen digestion (Kamalak *et al.*, 2005). The most common method up to date is to feed ruminants a diet that is high in by-pass proteins (Kung and Rode, 1996). Other methods may include treating the protein/AA source with heat or chemicals (treatment or modification, inhibition of proteolytic activity) or identifying proteins and AA's that are naturally protected (Kaufmann and Lupping, 1982). These methods can increase the availability of AA in the small intestine without increasing nitrogen losses (Kaufmann and Lupping, 1982).

2.4.5 Different methods to prevent rumen degradation

Amino acid supplementation through free AA is not recommended in the diets of ruminants, due to their rapid degradation in the rumen (Kamalak *et al.*, 2005). Thus, for free AA to pass in significant amounts to the small intestine a diet which excessively supersedes the requirements of the animal should be formulated (Kamalak *et al.*, 2005). This is, however, not always an economically feasible application. By chemical or physical alterations (encapsulation), these AA may become protected from rumen degradation. It has been shown that encapsulation can increase in the availability of a specific AA in the small intestine of the ruminant (Kung and Rode, 1996), thereby creating by-pass AA. The main problem associated with by-pass AA is whether or not the AA will be available in the small intestine for absorption and not completely pass through the entire digestive tract. Another consideration when by-pass AA are

incorporated into a feed, in either a pelleted form or in a total mixed ration (TMR), is the stability to endure the mechanical processing (pelleting) or the chemical compounds of other ingredients in the formulated diets.

2.4.5.1 Heat treatment

The rate of microbial fermentation can be reduced by reducing the solubility of the proteins through heat processing (Belits *et al.*, 2005). The heating proses cause carbonyl groups of sugars to combine with free amino groups and is known as the Maillard reaction (Belits *et al.*, 2005). This reaction causes the protein to be more resistant to the enzymatic hydrolysis of rumen microbes. Through heat treating, proteins form peptide links with asparagine and glutamine (Belits *et al*., 2005). These linkages are more resistant to enzymatic activity in the rumen.

Oilseed proteins are found to be the most economical to heat treat. However, heat-treatment of proteins may cause extensive damage to essential AA (Socha *et al.,* 2005). Essential AA includes lysine, methionine and cysteine (Kung and Rode, 1996). Heat treatment of proteins can also be used to increase the degradability of otherwise undegradable proteins (Robinson and McNiven, 1994; Prestlùkken, 1999). Unfortunately, if the heat processing is done too extensively with either too high temperatures or for a too long extended time, the acid detergent insoluble nitrogen content may increase (Broderick *et al.,* 1991). From this, we can conclude that moderate heat treatment may increase the flow of AA to the small intestine, but extensive heat treatment may damage essential AA or decrease the digestibility of proteins throughout the digestive tract.

2.4.5.2 Formaldehyde treatment

In previous years formaldehyde treatment of proteins was the most common method to prevent degradation of proteins in the rumen. This processing method was frequently used in the commercial sector where it has been exploited. Czerkawski (1986) explained that during this treatment process cross-links between the amino groups of protein forms and thus reduce the susceptibility of microbial degradation in the rumen. Other research (Kamalak *et al.*, 2005) showed that the formaldehyde treatment of high protein feedstuffs led to an increase in the number of proteins digested in the small intestine. They observed that the concentration of AA in the blood plasma was generally increased, but it depended on the tissue demands, as well as the supplied AA balance (Kamalak *et al.*, 2005).

Both the above-mentioned treatment methods may increase the percentage of proteins or AA in the small intestine and thus increase the availability of nutrients to the animal for production. However, excessive processing might have a negative effect on the digestion of the

microbial matter in the diet. Other research also indicated that over processing of proteins with formaldehyde had a negative effect on the nutritional value of the proteins when degradability was decreased (Manterola *et al.*, 2001). It can thus be concluded that this may also reduce the production of the animals.

2.4.5.3 Yeast cells

Previously, yeast cells had been used to supplement animal feed and improved dry matter intake, as well as milk production and milk composition, was reported with both dairy cows and goats (Dawson *et al.*, 1990). By examining the yeast cell it was found that the vacuoles of these cells accumulate AA like lysine and arginine in large amounts (Huber-Walchli and Wiemken, 1979). Ohsumi *et al.* (1994) research yeast cells and their ability to transport AA to the LDT as a non-chemical rumen-stable vector. These authors used lysine accumulating yeast cells and found that the accumulated lysine in the cells stayed intact during incubation in the *in vitro* procedure. When the yeast cells were incubated in the digestive enzyme solution, the accumulated lysine was completely released, proving that the yeast cells are effective as a rumen-stable AA source.

2.4.5.4 Protection of amino acids

Amino acid deficiency is expected to occur in high producing animals. This has resulted in several studies researching which AA are limiting production. Research indicated that methionine and lysine are the first limiting AA in high producing animals (Kung and Rode, 1996). Currently, various attempts have been made to developed and produce AA that prevents digestion in the rumen. These methods include structural manipulation to produce AA analogues and AA coated with resistant materials (Kamalak *et al.*, 2005).

There are various encapsulated analogues of AA that have been tested to withstand rumen degradation (Kamalak *et al.*, 2005). This includes methionine hydroxy analogues, mineral chelates, fat encapsulation and pH sensitive polymers. Methionine hydroxy analogues are one of the more tested encapsulated AA (Kung and Rode, 1996). The encapsulated AA consists of a core containing the specific AA and a pH sensitive coating that is synthetically produced (Kung and Rode, 1996). The capsule can thus resist degradation in the pH of the rumen but will be degraded at the lower pH of the LDT (Ohsumi *et al.*, 1994). Manterola *et al.* (2001) have indicated that increasing the amount of coating used in the encapsulation process may increase the effect of protection on the protein/AA source. The effect of these encapsulated AA has been widely evaluated in the dairy industry and resulted in increased production traits like milk yield and milk fat content. Encapsulation with fat, especially, is one of the methods that inspired several researchers. This is due to fat being an energy source, besides protecting AA from

rumen degradation that could be important in e.g. high yielding cows where extra energy may be used during the first stage of lactation (Kamalak *et al.*, 2005).

The most common encapsulated AA are methionine and lysine (Kung and Rode, 1996). These AA can also be protected from the enzymatic activity in the rumen through chemical or mechanical methods. Manufacturing of these protected/encapsulated AA has led to commercial product development in the animal feed industry. The different coatings used to produced encapsulated AA include polymeric compounds, fat, formulized proteins, a mixture of fat and proteins, mixtures of fat and calcium salts, and calcium salts of long chain fatty acids (Kamalak *et al.*, 2005).

The use of these encapsulated AA, especially methionine and lysine, has been evaluated in the dairy industry due to them being the first limiting AA in lactating cows (Kincaid and Cronrath, 1993). Despite variable responses in milk yield, slight positive increases in milk protein production were also observed (Kincaid and Cronrath, 1993). Overall, we can conclude that supplementing the diet with encapsulated AA may result in an increase in production, but unfortunately, the effect on wool production is still uncertain.

2.4.6 Advantages of rumen protection

The utilization of nutrients post-rumen has advantages for the animal. For example, energy losses that are related to the fermentation in the rumen may be reduced and the loss of protein sources when dietary proteins are transformed to MP may also be reduced. It must be taken into consideration that the protein digestive process in a ruminant may not be as efficient as in a monogastric animal when the site of absorption of nutrients are changed, from the rumen to the small intestine (Kamalak *et al.*, 2005). Another advantage of protecting AA is that the percentage of free AA to be used for production is higher in the small intestine of the ruminant. When supplementing AA in the diet of ruminant animals through the use of by-pass AA, consideration to the limiting AA associated with the particular physiological status or production of the animal will be required (Chalupa, 1975).

Despite these advantages associated with the use of rumen undegradable proteins or by-pass AA, there is still some considerable lack of responses seen in research. This can be due to different factors (Schingoethe, 1996):

- The first may be that the higher degree of by-pass of proteins/AA through the rumen could result in a negative effect on MP synthesis.
- The second factor could be that digestion of these by-pass protein/AA sources is poor in the small intestine and they pass through the entire digestive tract.
- A third factor could be that the proteins/AA that by-pass to the small intestine are inefficient to the limiting AA requirement for production.

2.5 Lysine influence

Meeting the requirement of a specific AA by means of protein supplementation may lead to an unnecessary provision of a less limiting AA (Galbraith, 2000) because a balanced mixture of essential AA is required for optimal wool growth rates (Khan *et al.*, 2012). Nonetheless, with this in mind, the reason for the use of lysine in a protein supplement is that it has an important role in protein synthesis in the inner root sheath of the wool follicle (Brusch, 2012). This is the area where fibre growth starts (Rogers, 1964). The high content of lysine in histone proteins, active in cell division, may be a reason for the effect of lysine on wool growth (Brusch, 2012). Unfortunately, there is little research done on the effect of lysine on wool growth.

2.6 Current use of synthetic amino acids

One of the most costly nutrients to include in animal feed is protein (Hynd *et al.,* 2015). Formulations used are usually set to include the least amount of crude protein (CP) not only to reduce the cost of the feed but to avoid over-providing AA and later N-excretion and pollution. Over-provision of AA might still occur when different sources of proteins (plant or animal) are used in the diet. The higher demand for crop yield with the reduction of nutrients and the unavailability of farmable land has led to the development of synthetic AA (Vieira *et al.*, 2016). Synthetic AA can thus be used to satisfy the ideal AA profile (Vieira *et al.*, 2016).

Utilizing these AA to satisfy the ideal AA profile of specific high producing animals can allow for more precision when formulating. This would provide better-balanced diets for specific production purposes that may result in improved performance (Schwab and Broderick, 2017). Unfortunately, synthetic AA cannot replace the entire CP to reduce feed costs, as this can lead to a loss in production. The level to which the CP content can be reduced would depend on the next limiting AA in the ideal AA profile, which is influenced by the inclusion of different protein sources in the formulated diet (Vieira *et al.*, 2016). However, as the CP levels of the diet are reduced with the use of synthetic lysine, the ratio of lysine to total AA are improved, leading to a decrease in unnecessary excretion of N and a decrease in feed cost (Miranda *et al.*, 2015).

These synthetic AA are already widely used in the monogastric animal feed industry due to the competitive nature of these production systems. In ruminants however, these AA are degraded in the rumen and thus form part of the MP of the rumen (Kung and Rode, 1996). The most common synthetic AA used in any production system are lysine and methionine as they are the first two limiting AA in the ideal AA profile (Miranda *et al.,* 2015).

2.7 Application

Little research has been done not only on the use of synthetic AA in ruminants, but more so on wool producing sheep. The purpose of this study will, therefore, be to determine whether or not

rumen-protected lysine could improve the wool production of Merino sheep. More so the wool growth, thus the FL, FD, SS, FC and CFW, through supplementing the diet of sheep with high and low levels of by-pass lysine and rumen degradable lysine. From the literature, it is expected that the highest response will be achieved in the treatment diet with the high levels of by-pass lysine. This is due to the absorption of adequate amounts of these limiting AA in the LDT.

2.8 References

Allden, W., 1979. Feed intake, diet composition and wool growth. In P. R. JL Black (Ed.), Physiological and environmental limitations to wool growth, 61–78.

Annison, E.F., 1981. The role of protein which escapes ruminal degradation. (Recent Advantages in Animal Nutrition in Australia). (D. J. Farrell, Ed.) (Unit). Armidale: University of New England Publishing.

Belits, H.D., Grosch, W. and Schieberle, P., 2005. Reaction involved in food chemistry. In: Food Chemistry. (Springer Berlin, Ed.) (3rd Ed.). Heidelberg, New York.

Benavides, M.V. and Maher, P., 2003. Genetic parameters of wool colour and skin traits in Corriedale sheep. Genetic and Molecular Biology, 26(3), 267–274.

Black, J.L. and Reis, P.J., 1979. Speculation on the control of nutrient partition between wool growth and other body functions. Armidale, NSW: Armidale, NSW, University of New England Publishing Unit.

Broderick, G.A., Wallace, R.J. and Ørskov, E.R., 1991. Control of Rate and Extent of Protein Degradation. In K. T., Tsuda, Y., Sasaki, R. (Ed.), Physiological Aspects of Digestion and Metabolism in Ruminants (CA, pp. 541–592). London: Academic Press Ltd.

Brusch, H., 2012. Histones and other nuclear proteins. Elsevier.

Campbell, A.J.D., Larsen, J.W.A. and Vizard, A.L., 2011. The effect of annual shearing time on wool production by a spring-lambing Merino flock in south-eastern Australia. Animal Production Science, 51, 939–951.

Chalupa, W., 1975. Rumen bypass and protection of proteins and amino acids. Journal of Dairy Science, 58(8), 1198–1218.

Collins, J.D. and Chaikin, M., 1968. Structural and non-structural effects in the observed stress-strain curve for wet wool fibres. Journal of the Textile Institute, 59(8), 379–400.

Corbett, J.L., 2001. Variation in wool growth with physiological state. Armidale: Univ. New England Publishing Unit.

Cottle, D.J. and Cowie, A.L., 2016. Allocation of greenhouse gas production between wool and meat in the life cycle assessment of Australian sheep production. Int. J Life Cycle Assess, 21, 820–830.

Czerkawski, J.W., 1986. An introduction to rumen studies (1st Ed.). Oxford, Oxfordshire: Pergamon Press.

Dawson, K.A., Newman, K.E. and Boling, J.A., 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. J. Anim. Sci, 68(10), 3392–3398.

De Barbieri, I., Hegarty, R.S., Li, L. and Oddy, V.H., 2015. Association of wool growth with gut metabolism and anatomy in sheep. Livestock Science, 173, 38–47.

Dyer, J.M., Bringans, S.D., Aitken, G.D., Joyce, N.I. and Bryson, W.G., 2007. Extraction and characterisation of non-scourable chromophores from discoloured fleece wool. Coloration Technology, 123(1), 54–58.

Everitt, G.C. and Taplin, D.E., 1967. The influence of prenatal nutrition on postnatal performance of merino lambs. Editorial Services Limited. Wellington.

Friend, M.A. and Robards, G.E., 2006. Wool production and quality of three strains of Merino in a semi-arid environment under different grazing strategies. Australian Journal of Experimental Agriculture, 46, 37–44.

Froidmont, E. and Bartiaux-Thill, N., 2004. Suitability of lupin and pea seeds as a substitute for soybean meal in high-producing dairy cow feed. Anim. Res, 53, 475–487.

Galbraith, H., 2000. Protein and sulphur amino acid nutrition of hair fibre-producing Angora and Cashmere goats. Livestock Production Science, 64, 81–93.

Huber-Walchli, V. and Wiemken, A., 1979. Differential extraction of soluble pools from the cytosol and the vacuoles of yeast (Candida utilise) using DEAE-dextran. Archives of Microbiology, 120(2), 141–149.

Huisman, A.E. and Brown, D.J., 2008. Genetic parameters for body weight, wool and disease resistance and reproduction traits in merino sheep. 2. Genetic relationships between body weight traits and other traits. Australian Journal of Experimental Agriculture, 48, 1186–1193.

Hynd, P.I., Edwards, N.M., Hebart, M., Mcdowall, M. and Clark, S., 2009. Wool fibre crimp is determined by mitotic asymmetry and position of final keratinisation and not ortho- and para-cortical cell segmentation. The Animal Consortium, 3(6), 838–843.

Hynd, P.I., Edwards, N.M., Weaver, S., Chenoweth, K., Stobart, R. and Heberle, N., 2015. Biological defleecing: intravenous infusion of amino acid mixtures lacking lysine and methionine creates a weakened zone in the wool staple, which is amenable to mechanical wool harvesting. Animal Production Science, 55, 1264–1271.

Kamalak, A., Canbolat, O., Gurbuz, Y. and Ozay, O., 2005. Protected Protein and Amino Acids in Ruminant Nutrition. Journal of Science and Engineering, 8(2), 84–88.

Kaufmann, W. and Lupping, W., 1982. Protected proteins and protected amino acids for ruminants. (E.L. Muller, I.H. Pike, & A.J.H. Vanes, Eds.), Protein Contribution of Feedstuffs for Ruminants (UK). London: Butterworth's.

Khan, M.J., Abbas, A., Ayaz, M., Naeem, M., Akhter, M.S. and Soomro, M.H., 2012. Factors affecting wool quality and quantity in sheep. Afr. J. Biotechnol, 11(73), 13761–13766.

Kincaid, R.L. and Cronrath, J.D., 1993. Effects of added dietary fat and amino acids on performance of lactating cows. Journal of Dairy Science, 76(6), 1601–1606.

Kuffner, H. and Popescu, C., 2012. Wool fibres - 8. (R. M. Koslowski, Ed.) (1st Ed.). Cambridge: Woodhead Publishing.

Kung Jr.L. and Rode, L.M., 1996. Amino acid metabolism in ruminants. Animal Feed Science Technology, 59, 167–172.

Lee, G.J. and Atkins, K.D., 1995. Consequences of reproduction on the live weight, fat depth, wool growth, and intake of grazing Merino ewes in the short and long term. Australian Journal of Experimental Agriculture, 35(2), 153–159.

Li, L., Oddy, V.H. and Nolan, J.V., 2008. Whole-body protein metabolism and energy expenditure in sheep selected for divergent wool production when fed above or below maintenance. Australian Journal of Experimental Agriculture, 48, 657–665.

Manterola, H.B., Cerda, D.A. and Mira, J.J., 2001. Protein degradability of soybean meal coated with different lipid substances and its effects on ruminal parameters when included in steer rations. Animal Feed Science and Technology, 92, 249–257.

Marshall, R.C., Orwin, D.F.G. and Gillespie, J.M., 1991. Structure and biochemistry of mammalian hard keratin. Electron Microsc. Rev, 4, 47–50.

Matebesi, P.A., Van Wyk, J.B. and Cloete, S.W.P., 2009. Genetic parameters for subjectively assessed wool and conformation traits in the Tygerhoek Merino flock. South African Journal of Animal Science, 39(3).

Mcgregor, B.A., De Graaf, S.P. and Hatcher, S., 2016. On-farm factors affecting physical quality of Merino wool. 1. Nutrition, reproduction, health and management. Small Ruminant Research, 137, 138–150.

Meissner, H.H. and Todtenhofer, U., 1998. Influence of casein and glucose or starch supplementatio in the rumen or abomasum on utilization of hay by sheep. S. Afr. J. Anim. Sci, 19(1), 43–49.

Miranda, D.J.A., Vieira, S.L., Favero, A., Angel, C.R., Stefanello, C. and Nogueira, E.T., 2015. Performance and meat production of broiler chickens fed diets formulated at different crude protein levels supplemented or not with L-valine and L-isoleucine. Anim. Feed. Sci. Technol, 206, 39–47.

Molina Alcaide, E., Yáñez Ruiz, D.R., Moumen, A. and Martín García, A.I., 2003. Ruminal degradability and in vitro intestinal digestibility of sunflower meal and in vitro digestibility of olive by-products supplemented with urea or sunflower meal Comparison between goats and sheep. Anim. F. Sci. Technol., 110, 3–15.

Morley, F.H.W., 1982. Managing grazing systems. In Morley, F.H.W. (Ed). Grazing Animals. Agricultural Systems (Vol. 8).

Mulrooney, C.N., Schingoethe, D.J., Kalscheur, K.F. and Hippen, A.R., 2009. Canola meal replacing distillers' grains with soluble for lactating dairy cows. J. Dairy Sci., 92, 5669–5676.

Nancarrow, M.J., Nagorcka, B.N. and Purvis, I.W., 1998. Kin and follicle attributes contributing to differences in clean fleece weight in superfine Merino sheep. Anim. Prod. Aust., 22, 253–256.

Norris, B.J., Colditz, I.G. and Dixon, T.J., 2008. Fleece rot and dermatophilosis in sheep. Vet Microbial, 128(3–4), 217–230.

Ohsumi, T., Sato, H., Yoshihara, Y. and Ikeda, S., 1994. Selection and Breeding of Lysine-accumulating Saccharomyces cerevisiae as a Stable Source of Lysine in the Rumen. Biosci. Biotechnol. Biochem. 58, 1302–1305.

Pierzchala-Koziec, K., Kepys, B., Oeltgen, P. and Scanes, C.G., 2018. Developmental Changes in the Pituitary-Adrenocortical Axis and Plasma Met-Enkephalin Concentration in Response to Isolation Stress in Growing Lambs. Folia Biologica (Kraków), 66(2), 53–61.

Popescu, C. and Höcker, H., 2007. Hair-the most sophisticated biological composite material. Chemical Society Reviews, 36, 1282–1291.

Prestlùkken, E., 1999. In situ ruminal degradation and intestinal digestibility of dry matter and protein in expanded feedstuffs. Animal Feed Science and Technology, 77, 1–23.

Ramada, W.A., El-Harairy, M.A., Khalil, W.A. and Youssef, A.H., 2017. Impact of Adding Rumen Protected Lysine or / and Methionine on Some Wool Characteristics in Barki Sheep. J. Anim. and Poultry Pod. 8(7), 173–177.

Reis, P. J., 2000. Effects of amino acids on the growth and properties of wool. Armidale: Univ. New England Publishing Unit.

Reis, P.J., Funks, D.A. and Munro, S.G., 1992. Effects of Abomasal Protein and Energy Supply on Wool Growth in Merino Sheep. Aust. J. Agric. Res, 43, 1353–1366.

Robertson, S.M., Robards, G.E. and Wolfe, E.C., 1996. Factors contributing to the variation in staple strength of reproducing ewes. Anim. Prod. Aust, 21, 166–169.

Robinson, P.H., Chalupa, W., Sniffen, C.J., Julien, W.E., Sato, H. and Watanabe, K., 1998. Ruminally Protected Lysine or Lysine and Methionine for Lactating Dairy Cows Fed a Ration Designed to Meet Requirements for Microbial and Postruminal Protein 1. Journal of Dairy Science, 81, 1364–1373.

Robinson, P.H. and McNiven, M.A., 1994. Influence of flame roasting and feeding frequency of barley on performance of dairy cows. Journal of Dairy Science, 77(12), 3631–3643.

Rogers, G.R. 1964. Isolation and properties of inner sheath cells of hair follicles. Experimental Cell Research (Vol. 33).

Safari, E., Fogarty, N.M. and Gilmour, A.R., 2005. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science, 92, 271–289.

Savary, I.C., Hoskin, S.O., Dennison, N. and Lobley, G.E., 2001. Lysine metabolism across the hindquarters of sheep; effect of intake on transfers from plasma and red blood cells. British Journal of Nutrition, 85, 565–573.

SCA., 1990. Feeding standards for Australian livestock. Ruminants. Australian Agriculture Council. Ruminants Subcommittee.

Schingoethe, D.J., 1996. Balancing the amino acid needs of the dairy cow. Animal Feed Science Technology, 60, 153–160.

Schwab, C.G. and Broderick, G.A., 2017. A 100-Year Review: Protein and amino acid nutrition in dairy cows. J. Dairy Sci, 100, 10094–10112.

Socha, M.T., Putnam, D.E., Garthwaite, B.D., Whitehouse, N.L., Kierstead, N.A., Schwab, C.G. and Robert, J.C., 2005. Improving intestinal amino acid Supply of pre-and postpartum dairy cows with rumen-protected methionine and lysine. Journal of Dairy Science, 88, 1113–1126.

Thompson, A.N., Ferguson, M.B., Gordon, D.J., Kearney, G.A., Oldham, C.M. and Paganoni, B.L., 2011. Improving the nutrition of Merino ewes during pregnancy increases the fleece weight and reduces the fibre diameter of their progeny's wool during their lifetime and these effects can be predicted from the ewe's live weight profile. Animal Production Science, 51, 794–804.

Thompsona, R.L. and Callinan, A.P., 1981. The Effects of Nematodiasis on Weaner Sheep in Western Victoria. Aust. J. Agric. Res (Vol. 32).

Toledo, J.B., Furlan, A.C., Pozza, P.C., Carraro, J., Moresco, G., Ferreira, S.L. and Gallego, A.G., 2014. Reduction of the crude protein content of diets supplemented with essential amino acids for piglets weighing 15 to 30 kilograms. R. Bras. Zootec, 43(6), 301–309.

Vieira, S.L., Stefanello, C. and Cemin, H.S., 2016. Lowering the dietary protein levels by the use of synthetic amino acids and the use of a mono component protease. Anim. Feed Sci. Technol., 221, 262–266.

Wang, H., Mahar, T., Liu, X., Swan, P. and Wang, X., 2011. Modelling of fibre-diameter-dependent light scattering to determine diameter corrections for colour measurement of wool. The Journal of the Textile Institute, 102(12), 1031–1043.

Waters, C.M., Coelli, K.A., Lee, G.J. and Atkins, K.D., 2000. Reproduction effects on annual fleece production, live weight and body condition of grazing Merino ewes. Australian Journal of Experimental Agriculture, 40, 931–937.

Williams, A.J. and Butt, J., 1989. Wool growth of pregnant Merino ewes fed to maintain maternal live weight. Australian Journal of Experimental Agriculture, 29(4), 503–507.

Williams, A.J. and Winston, R.J., 1987. A Study of the Characteristics of Wool Follicle and Fibre in Merino Sheep Genetically Different in Wool Production. Aust. J. Agric. Res (Vol. 38).

Williams A.J., 2000. Speculations on the biological mechanisms responsible for genetic variation in the rate of wool growth. Armidale: Univ. New England Publishing Unit.

Zargorakis, K., Liamadis, D., Milis, C., Dotas, V. and Dotas, D., 2018. Effects of replacing soybean meal with alternative sources of protein on nutrient digestibility and energy value in sheep diets. S. Afr. J. Anim. Sci., 48(3), 488–496.

Chapter 3:
The influence of a commercial protected lysine on production parameters in Merino rams

3.1 Abstract

The purpose of this study was to determine whether a synthetic amino acid could improve the wool production of Merino sheep. More so the wool growth, fibre length (FL), fibre diameter (FD), staple strength (SS), fibre curvature (FC) and clean fleece weight (CFW), by supplementing the diet with high and low levels of by-pass lysine and rumen degradable lysine (2x2 factorial design). Forty Merino rams with an age of 1.5 years and average weight of 37kg, were fed for 3 months and during this period the feed intake were measured daily to calculate the average daily intake (ADI). The sheep were weighed weekly to calculate the average daily gain (ADG). Blood samples were collected at the beginning of the trial and once during the trial to determine the lysine content of the plasma. At the end of the trial all the rams were sheered to determine the wool production parameters throughout the trial. Results indicated that the supplementation of By-pass amino acid (AA) had a significant ($P \leq 0.05$) influence on the dry matter intake (DMI) of the rams. Wool parameters were, however, not affected by treatment. The blood plasma lysine levels were only significantly higher ($P \leq 0.05$) for the diets high in lysine but were not affected by the degradability. It was concluded that the use of rumen protected lysine as a supplementation in Merino rams showed little responses to increase the production parameters.

3.2 Introduction

Wool is a large export commodity in South Africa where the price is determined by supply and demand (Dlodlo *et al.*, 2009). It has dynamic versatility and is used in numerous sectors ranging from fashion clothing, flooring, architecture and even medical use (Kuffner and Popescu, 2012). The average price per kilogram for the 2017/2018 wool clip season was on average R183.84 (top lines). This price has increased by approximately R30/kg (16.3%) in just one clip season (Cape Wools SA, 2019). Therefore, improving the production of wool or optimizing the cost of the production of wool is of importance to the economic contribution of this farming sector.

When the composition of wool is considered it is classified as a polymer consisting of amino acids (AA) (Kuffner and Popescu, 2012). As a result most of the research done on wool growth had a nutritional basis and when AA in the diets were considered it mostly included sulphur containing AA (Ramada *et al.*, 2017) and the key to these studies was to increase wool

yield. According to Safari *et al.* (2005), several parameters within wool contribute to wool yield in the sheep namely, staple length (SL), staple strength (SS), clean fleece weight (CFW), fibre diameter (FD) and fibre curvature (FC).

When the individual wool parameters are considered as the staple length (SL) it was stated that it is a function of individual fibre lengths as well as the extent of the crimping of the fibre (Khan *et al.*, 2012). It was also shown that SL is phenotypically directly correlated to the live weight of the sheep (Huisman and Brown, 2008). Mcgregor *et al.* (2016) reported that follicle density in sheep with a larger frame size or live weight may have a larger skin surface area. This then results in fibres with larger diameters and thus a higher fibre and fleece mass (Mcgregor *et al.*, 2016). Fibre diameter (FD) is the wool parameter that is one of the most important determinants of the price of raw wool. In general finer wool with a lower FD will receive a higher price (Friend and Robards, 2006). Vizard and Williams (1993) in contrast emphasized that an increase in FD results in an increase in CFW.

A major objective in any animal production system is to increase the efficiency of converting nutrients into animal products (De Barbieri *et al.*, 2015). It is not possible to distinguish between the effects of nutrition on wool growth without it influencing the growth of the animal. Earlier research by Allden (1979), shows that wool parameters e.g. FD, change due to an increase or decrease or the quality of the nutrient availability. Khan *et al*. (2012) also stated that a variation in nutrient supply to the wool follicle can have a considerable influence on not only the rate of fibre production but also the different characteristics of the fleece. According to the latter authors nutritional level will thus affect also the live weight of the sheep. A study done by Galbraith (2000) suggested that the fibre growth of sheep may be limited by protein content that could be inadequate in the diet. Their study also reported that additional protein supplementation can influence wool yield, but on the negative side, it increases FD.

Changes in the fractional protein synthesis rate and the total protein synthesis in the skin are associated with increased wool growth (Li *et al.*, 2008). Allden (1979) further reported that when rumen degradation of proteins is limited in sheep, substantial increases in the growth rate of the wool are possible. Microbial protein (MP) alone is likely to meet the maintenance requirements of an animal (Kamalak *et al.,* 2005), but it is insufficient to meet the demand for optimal production of the animal (Kaufmann and Lupping, 1982). The quantity of AA that escapes degradation in the rumen is dependent on factors like, AA solubility and the rate of passage to the small intestine (Kamalak *et al.*, 2005). Furthermore, Savary *et al.* (2001) showed that increased intake of rumen-protected lysine is linearly correlated with the absorption of these AA in the lower digestive tract (LDT). A variety of methods and techniques have been developed in history to increase the supply of AA to the small intestine. By decreasing the AA retention times in the rumen, the rumen degradation of these AA can be reduced (Kung and Rode, 1996). The most common practice, however, is to feed ruminants a diet high in by-pass proteins (Kung and Rode, 1996).

Research indicated that it is generally methionine and lysine that is the first limiting AA for production in animals (Kung and Rode, 1996). Currently various attempts have been made to developed and produce AA that prevents degradation in the rumen. These methods include structural manipulation to produce AA analogues and AA coated with resistant materials (Kamalak *et al.*, 2005). The use of these encapsulated AA, especially methionine and lysine, has been widely evaluated in the dairy industry due to these AA being the first limiting AA in lactating cows. Despite variable responses in the milk yield, slight positive increases in milk protein production were observed with the increased addition methionine and lysine (Kincaid and Cronrath, 1993). Thus, supplementing the diet with encapsulated AA may lead to an increase in animal production, but unfortunately, the influence on wool production is still uncertain.

The reason for the use of lysine in a protein supplement is that it has an important role in protein synthesis that pertains to the inner root sheath of the wool follicle (Brusch, 2012). This is the area where fibre growth starts (Rogers, 1964). The high content of lysine in histone proteins, active in cell division, may be a reason for the effect of lysine on wool growth (Brusch, 2012). Unfortunately, there is little research done on the effect of lysine on wool growth. The purpose of this study was, therefore, to determine whether synthetic lysine, either protected or unprotected against ruminal degradation would improve the wool production of Merino sheep. More so the wool growth, thus the FL, FD, SS, FC and CFW, through supplementing the diet of these sheep with high and low levels of by-pass lysine and rumen degradable lysine. From the literature, we expected the highest response in the treatment diet with the high levels of by-pass lysine. This would be due to the absorption of these AA in the LDT.

3.3 Materials and Methods

All research work in this trial was conducted in agreement to the Stellenbosch University's Animal Ethics Committee (AUC-2018-6493).

3.3.1 Experimental Design

Forty 15-month-old Merino rams were selected from the flock at the Elsenburg Research Farm, Western Cape, South Africa. The rams were randomly selected to ensure a homogenous group according to an average weight of 37kg ± 2kg in each treatment group. A two- by- two factorial design was used to assess the inclusion of a high or low level of either rumen degradable lysine or by-pass lysine in a maintenance ram diet. The experimental design (Figure 3.1) resulted in four treatments with ten animals per treatment.

		Lysine Level	
		Low	High
Rumen Degradability	Rumen Degradable	LLU (n=10)	HLU (n=10)
	By-pass	LLU (n=10)	HLP (n=10)

Figure 3.1: Graphic representation of the experimental design.

Treatment 1 was a diet with a low level of rumen degradable lysine (LLU), Treatment 2 was a diet with a high level of rumen degradable lysine (HLU), Treatment 3 was a diet with low levels of by-pass lysine (LLP) and Treatment 4 was a diet with high levels of by-pass lysine (HLP). Treatment diets were mixed in separate batches and had a composition as seen in Table 3.1. The feed for the trail was provided by AFGRI Animal Feed.

3.3.2 Sampling

Forty merino rams received the respective treatment diets *ad libitum* for a period of 3 months from September to November 2018. Sheep were housed in individual pens in a sheep shed on slatted wooden floors. Prior to the start of the trial, the animals were acclimatized to the environment by feeding ground oat hay *ad libitum* for two weeks. This was also done to ensure a baseline level for amino acids to prevent any carry-over effects of feed before the onset of the trial. Thereafter, the animals were adapted on the four treatment diets for another 10 days before any measurements were recorded. During the subsequent three-month period the daily intake was recorded. The animals were fed in the morning (08h00) and the afternoon (16h00) with *ad libitum* access to feed. Every seven days the feed in the troughs of each animal was weighed back to determine the average daily intake.

Every seven days the sheep were weighed after their morning feeding. This was used to calculate the growth rate of each sheep over the three-month period. Average daily gain (ADG) was determined throughout the trial.

At the onset of the trial, as well as two months (Nolte and Ferreira, 2005) into the trial, blood was collected from all the animals at 08h00 before the morning feeding. Blood was extracted from the jugular vein into sterile 10 ml vacutainer blood collection tubes (K3 EDTA) and placed on ice to chill before processing.

After the three-month trial period, all the sheep were sheered to determine wool production during the trial period, all the rams entered the trial sheered. Greasy fleece weight was determined on the day of sheering by weighing the whole fleece of each animal. A midrib wool

sample of each sheep was sampled and send for wool parameter analyses at a commercial wool company.

Table 3.1 Feed and nutrient composition of the four different treatment diets (g/kg)

Description	LLU[8]	HLU[9]	LLP[10]	HLP[11]
Feed composition:				
Maize	249.8	249.7	249.8	249.7
Wheat Straw	200.0	199.9	200.0	199.8
Soya Hulls	118.0	117.9	118.0	117.9
Lucerne Hay	100.0	100.0	100.0	99.9
Wheat Bran	81.5	81.5	81.5	81.4
Maize gluten [1]	80.0	80.0	80.0	79.9
Molasses Syrup	50.0	50.0	50.0	50.0
Canola meal	37.0	37.0	37.0	37.0
Hominy Chop	32.5	32.5	32.5	32.5
Soy bean meal [2]	20.0	20.0	20.0	20.0
Limestone [3]	14.0	14.0	14.0	14.0
Min-Vit premix	7.0	07.0	07.0	7.0
Ammonium Sulphate	4.0	4.0	4.0	4.0
Coarse salt	3.2	3.2	3.2	3.2
Synthetic methionine [4]	3.0	3.0	3.0	2.9
Synthetic Lysine	0.05	0.55	/	/
Encapsulated Lysine [5]	/	/	0.075	0.80
Nutrient composition:				
Dry Matter	897.9	909.0	911.9	895.3
Ash	66.9	66.3	66.9	66.3
Crude Protein	135.7	137.7	135.6	138.1
Ether Extract	151.6	155.9	159.7	157.2
Crude Fibre	347.2	348.1	346.4	345.2
NDF[6]	202.8	202.2	203.4	203.1
ADF[7]	25.3	22.3	22.3	24.5

Retail name of raw materials are as follow [1] Gluten 20®, [2] Soy oilcake 47®, [3] Feed lime®, [4] Metasmart Dry®, [5] AminoCap L®, [6] Neutral Detergent Fibre, [7] Acid Detergent Fibre, [8] Low levels of Unprotected lysine, [9] High levels of Unprotected Lysine [10] Low levels of Protected Lysine, [11] High levels of protected Lysine.

3.3.3 Laboratory Analyses

3.3.3.1 Feed Analyses

All four TMR diets were sampled and ground through a 1 mm milling screen using a laboratory hammer mill (Scientec RSA Hammer mill Ser. Nr 372; Centrotec) to prepare samples for proximate analysis. Samples were stored in honey jars at 18ºC until analysed. To determine the

dry matter (DM) all the samples were placed in a forced-air oven at 105ºC for 24 hours. All proximate analyses of TMR diets were done according to the Association of official analytical chemists (2005) procedures. Procedure codes are 935.11 for CP, 920.39 for ether extract (EE), and 962.09 for crude fibre (CF). Analyses for NDF and ADF were according to Ankom procedures ($Ankom_{220}$ Fiber Analyser, ANKOM Technology, Fairport, NY, USA) as referenced by Goering and Van Soest (1970). Ash was determined by incineration in a furnace at 500ºC for 6 hours to determine organic matter (OM), calculated as the difference between total DM and ash (AOAC, 2005).

3.3.3.2 Blood Analyses

The blood was processed within one hour after collection for physiological AA analysis. Samples were centrifuged at 4500 rpm at 4 °C for ten minutes. The plasma, free of haemolysis and red blood cells, was then deproteinized using sulfosalicylic acid and an internal standard (L-Norvaline). The internal standard selected was due to the AccQ Tag method used during processing (Holtrop *et al.*, 2002). The samples were then vortexed and placed in a refrigerator for 1 hour before centrifuging at 13000rpm for 10 min at 4 ºC. Prior to the amino acid (AA) analysis one ml supernatant was pipetted into sterile 2ml Eppendorf tubes and stored in a -80ºC freezer until AA analysis (Holtrop *et al.*, 2002). The frozen samples were analysed at Labworld (Isando, South Africa). After samples were thawed, 10 µl of the deproteinized supernatant was derivatized using a Waters AccQ Tag Ultra Chemistry Kit (Waters Corporation, Milford, MA, USA) and analysed for free AA using ultra-performance liquid chromatography (UPLC) on a Waters ACQUITY UPLC H-Class System equipped with a UV detector (Hong *et al.,* 2009).

3.3.3.3 Wool Analyses

Wool samples were analysed by a commercial wool company in accordance with the Wool Testing Bureau of South Africa (WTB), licensed with the International Wool Textile Organization (IWTO). Measurement of the mean and distribution of FD of wool using an Optical Fibre Diameter Analyser (OFDA) (IWTO, 1998). The SL (kilotext) and SS (Newtons) were tested with the aid of the ATLAS machine following the IWTO-30 procedures (Bidinost *et al.*, 2008). The raw wool yield was determined as described by the IWTO-19 standard operating procedure (IWTO, 1998).

3.3.4 Statistical Analyses

A factorial ANOVA was used to compare the response variables versus the two factors, namely lysine [with levels High (HL) and Low (LL)] and protection [with levels unprotected (U) and protected (P)] using STATISTICA, version 13 (TIBCO Software Inc., 2017). With the non-

significance of interaction effects, the main effects of lysine and protection were interpreted individually.

The interaction effects were interpreted whether they were significant. These differences in interaction means were investigated with LSD multiple comparisons or ad hoc tests. When the respective interaction variances were non-homogeneous according to the Levene's test, the comparisons were done with a Games-Howell ad hoc tests. The residuals were checked for normality with normal probability plots and a Shapiro Wilk test. Significance was declared at $P \leq 0.05$ and a tendency at $P \leq 0.1$.

3.4 Results and Discussion

When all four diets (Table 3.2) were compared, a significant difference ($P < 0.05$) was observed among treatments regarding DMI where significant interactions also occurred. For the low lysine treatments, protection against ruminal degradation lowered DMI, but for the high lysine treatments protection increased DMI. The HLP treatment indicated a significantly higher ($P \leq 0.05$) average DMI compared to the other diets and the LLP treatment a significantly ($P \leq 0.05$) lower DMI. In this study, protection against ruminal degradation decreased DMI when the level of supplementation was low, but when it was high, protection increased DMI. These findings are in contrast to research done by Xue *et al.* (2011), where the dietary supplementation of rumen protected lysine did not affect intake in beef cattle. These authors, however, reported an increase in the ADG and an improvement in the FCR of beef cattle supplemented with protected lysine, which was not the case in the present stud.

When considering ADG, interactions among the four different diets were also observed. A tendency to differ ($P \leq 0.10$) was found for the ADG among treatments (Table 3.2), but not for FCR. The average reported FCR of the most effective breed (Dorpers) is between 4 and 5 on high concentrate diets in a feedlot environment (NRC, 2007; Wang *et al.*, 2016), 5 and 6 when sheep graze on high quality forages (Fahmy *et al.*, 1992), compared to more than 6 on low quality feeds (Malik *et al.*, 1996). The relatively high FCR value observed in the current study can be attributed to the use of a low-quality maintenance diet (Tables 3.1). The reason for the use of the selected experimental diets was only to evaluate protected and unprotected lysine and lysine inclusion levels on wool growth parameters, thus the focus was not on finishing.

Differences in the weight of animals in the treatment groups could have contributed to the lack of significant differences in ADG and FCR. During the trial it was found that the DMI of these animals stayed close to 4% of live weight (LW). For this reason, it was necessary to further observe the effect of inclusion levels and protection in Tables 3.3 and 3.4, respectively.

Table 3.2 Production parameters of Merino sheep fed four different diets with either high or low levels of encapsulated and unprotected lysine

Production parameter	**LLU**[1]	**LLP**[2]	**HLU**[3]	**HLP**[4]	***P***[8]
DMI[7] (kg/day)	2.65[ab] ± 0.03	2.48[c] ± 0.06	2.51[bc] ± 0.07	2.71[a] ± 0.06	0.002
ADG[5] (kg)	0.34 ± 0.02	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.081
FCR[6] (kg DM/kg gain)	8.07 ± 0.43	8.86 ± 0.38	8.38 ± 0.32	8.78±0.10	0.554

[ab] Mean within rows with different superscripts differ ($P \leq 0.05$).
[1] Low level unprotected lysine, [2] Low level encapsulated lysine, [3] High levels unprotected lysine and [4] High levels encapsulated lysine.
[5] Average daily gain of sheep during sheep trial.
[6] Feed conversion ratios of sheep during the trial period.
[7]Dry Matter Intake of sheep during the trial period.
[8] *P*-value when considering One-way Anova.

Table 3.3 indicates that none of the ADG or FCR production parameters for levels of lysine inclusion in the feed showed any differences. As both ADG and FCR are dependent on DMI (Ocak *et al.*,2016), the absence of DMI differences, when considering only the inclusion level (LL and HL) (Table 3.3), maybe the greatest cause for not seeing any differences in both ADG and FCR.

However, when the results of Table 3.4 are compared, a tendency was observed ($P \leq 0.10$) for the ADG to differ between treatments. When comparing FCR between treatments a tendency ($P \leq 0.10$) towards a higher FCR value was observed for the protected lysine treatment. The lack of treatment response regarding DMI (Table 3.4) is in contrast to the response seen in some dairy studies were the inclusion of protected lysine led to a decrease in DMI of the animals (Patton, 2010). In the current study, it appeared that the protected lysine product did not improve animal responses.

Table 3.3 Production parameters of Merino sheep receiving diets with low and high inclusion levels of synthetic lysine

Production parameter	**LL**[1]	**HL**[2]	***P***
DMI[5] (kg/day)	2.56 ± 0.04	2.61 ± 0.05	0.414
ADG[3] (kg)	0.31 ± 0.01	0.31 ± 0.01	0.715
FCR[4] (kg DM/kg gain)	8.46 ± 0.29	8.58 ± 0.17	0.724

[1] Low and [2] High inclusion levels of synthetic or encapsulated lysine.
[3] Average daily gain of sheep during the trial period.
[4] Feed conversion ratio of sheep during the trial period.
[5] Dry Matter Intake of sheep during the trial period.

Table 3.4 Production parameters of Merino sheep receiving diets with unprotected lysine and protected lysine

Production parameter	Unprotected[1]	Protected[2]	*P*
DMI[5] (kg/day)	2.58 ± 0.04	2.60 ± 0.05	0.735
ADG[3] (kg)	0.32 ± 0.01	0.30 ± 0.01	0.099
FCR[4] (kg DM/kg gain)	8.22 ± 0.26	8.81 ± 0.19	0.081

[1]Unprotected synthetic lysine and [2] Encapsulated commercial lysine.
[3] Average daily gain of sheep during the trial period.
[4] Feed conversion ratio of sheep during the trial period.
[5] Dry Matter Intake of sheep during the trial period.

Results on wool parameters are presented in Tables 3.5, 3.6 and 3.7. For most of the wool parameters the tests for interaction were not significant, except for MFD (Table 3.5) where the interaction had a tendency to differ, meaning that the protected and unprotected lysine means behaved consistently over the levels of lysine inclusion. Thus, the main effects lysine inclusion and lysine protection may be interpreted (Table 3.6 and 3.7). However, the interaction means (Table 3.5) may also be interpreted using the LSD test and are sometimes more interesting than comparing the main effects themselves. The significant differences among the interaction means are identified with superscripts in Table 3.5 and discussed below.

The treatment HLU differed significantly ($P \leq 0.05$) from the LLU treatment and had the highest MFD value, while LLU had the lowest MFD value. However, no significant differences were observed when considering the treatments with protected lysine in the diet. Other wool parameters that showed interactions were SD, Yield and Staple length. When comparing the means of SDFD, it was determined that the treatment LLU and HLU differed significantly ($P \leq 0.05$). The HLU treatment had the highest SDFD value. The protected lysine treatments did not differ from the unprotected treatments (Table 3.5). From Table 3.5 it is clear that the HLU treatment differed significantly from both the LLU and LLP treatment when yield was analysed. The HLU showed the highest clean fleece yield. Staple length also revealed that the HLU treatment differed significantly ($P \leq 0.05$) from the LLP treatment and had the highest mean while LLP revealed the lowest mean.

Mean averages for MFD of below 20.1 µm for all treatments (Table 3.5) indicate that all the wool of the treatments can be classified as fine wool (Cape Wools SA, 2019). The April 2019 price difference between 20 µm wool and 18.5 µm wool was R7.17/kg (Cape Wools SA, 2019). Although the differences for the MFD values in Table 3.5 only showed a tendency to differ significantly, the difference between the HLU and LLU treatments were more than 1 µm. From a commercial farmer point of view, the difference in income will be marginal. When examining the other wool parameters where interactions were observed, there were no

differences between protected and unprotected lysine supplementation at either the high or low levels of inclusion.

Table 3.5 Wool parameters of Merino sheep of four different diets with either high or low levels of encapsulated and unprotected lysine.

Wool parameters	LLU[1]	LLP[2]	HLU[3]	HLP[4]	*P*[8]
MFD[5] (µm)	18.97[b] ± 0.35	19.49[ab] ± 0.54	20.64[a] ± 0.52	19.55[ab] ± 0.45	0.0950
C of V[6] (%)	18.4 ± 0.87	18.8 ± 0.62	19.94 ± 0.76	19.57 ± 0.76	0.614
SDFD[7](µm)	3.49[b] ± 0.19	3.67[ab] ± 0.16	4.13[a] ± 0.22	3.81[ab] ± 0.15	0.180
Comfort factor (%)	99.22 ± 0.34	98.82 ± 0.43	97.4 ± 1.18	99.04 ± 0.26	0.134
Yield (%)	71.59[b] ± 1.49	72.66[b] ± 1.30	76.6[a] ± 0.77	74.35[ab] ± 1.24	0.185
Crimp	10 ± 0.33	10.9 ± 0.28	9.5 ± 0.48	10.1 ± 0.48	0.712
Staple length (cm)	60.5[ab] ± 1.34	53.8[b] ± 1.79	61.2[a] ± 1.22	56.6[bc] ± 1.50	0.482
Strength (N/ktex)	47.9 ± 2.15	41.9 ± 3.37	38.5 ± 4.17	39 ± 5.24	0.485
Tip	51.2 ± 13.50	51.9 ± 9.73	45 ± 15.07	20.5 ± 10.47	0.359
Middle	48.8 ± 13.50	48.1 ± 9.73	54 ± 15.43	78.5 ± 10.30	0.319
Greasy Fleece weight	3.24 ± 0.16	3.07 ± 0.17	3.06 ± 0.17	3.08 ± 0.18	0.576

[ab] lettering to identify significant interactions means ($P < 0.05$) when considering LSD test.
[1] Low level unprotected lysine, [2] Low level encapsulated lysine, [3] High levels unprotected lysine and [4] High levels encapsulated lysine
[5] Mean fibre diameter
[6] Coefficient of variation of fibre diameter
[7] Standard deviation of fibre diameter
[8] *P*-value when considering One-way Anova

Table 3.6 indicates the wool parameter means of the different levels of lysine in the diets. A significant difference ($P \leq 0.05$) was observed among treatments regarding SDFD and wool yield. The wool measured from the animals that received diets with high lysine levels (HL) had the greater SDFD ($P \leq 0.05$) and yield percentage ($P \leq 0.1$) compared to the LL treatments. The standard deviation of fibre diameter is an indication of the diameter deviations on both sides of the average fibre diameter (Botha and Hunter, 2010). A lower SDFD value indicates that measured wool samples had a more uniform FD. The treatment means of LL showed an SDFD of 3.58 ± 0.12 µm. This indicates that 68% of the FD values for these treatments were between 15.65 µm and 22.81 µm. The HL treatments in comparison had means for SDFD of 3.97 ± 0.14 µm and this indicated that 68% of the FD values for these treatments were between 16.31 µm and 24.07 µm. As indicated by Hunter *et al.* (1985) the FD distribution of fine wool tends to be more peaked and skewed than in coarser wools where the distribution tends to be more symmetrical (Botha and Hunter, 2010). These findings correspond with the results in Table 3.6 was the LL-treatments had a tendency ($P \leq 0.07$) towards lower SDFD and a smaller SDFD ($P \leq$

0.04); indicating a normal distribution that is more peaked. The corresponding responses were seen for treatments HL where a higher FD was measured and a bigger SDFD was calculated.

The greater ($P \leq 0.01$) yield percentage (Table 3.6) for the treatments with a high level of lysine might be due to the higher amount of lysine absorbed from the high lysine content diets. Rogers (1964) proved that protein synthesis that occurs in the inner root sheath of the wool follicle is largely influenced by the lysine content present in these cells. The inner root sheath was found to be the area where fibre growth commences (Rogers, 1964). The greater lysine absorption from diets with higher lysine content could also have increased the presence of histone proteins, high in lysine, with their active cell division and thus increasing the total wool growth and subsequently the wool yield (Brusch, 2012). An increase in MFD was found to increase the CFW of the wool (Vizard and Williams, 1993). Thus, in accordance with the significant difference found in the wool yield between the treatments, LL and HL, the MFD values of the treatments with the higher lysine content were also found to be greater in this study. None of the balance of the wool parameters showed any significant differences between the treatments.

Table 3.6 Wool parameters of Merino sheep receiving diets with low and high inclusion levels of synthetic lysine.

Wool parameters	**LL**[1]	**HL**[2]	***P***
C of V[4] (%)	18.6 ± 0.52	19.76 ± 0.53	0.136
SDFD[5]	3.58 ± 0.12	3.97 ± 0.14	0.040
Comfort factor (%)	99.02 ± 0.27	98.22 ± 0.62	0.237
Yield (%)	72.125 ± 0.97	75.48 ± 0.76	0.010
Crimp	10.45 ± 0.23	9.80 ± 0.34	0.115
Staple length	57.15 ± 1.33	58.90 ± 1.08	0.244
Strength	44.90 ± 2.06	39.25 ± 3.26	0.156
Tip	51.55 ± 8.10	32.75 ± 9.36	0.138
Middle	48.45 ± 8.10	66.25 ± 9.46	0.162
Greasy fleece weight	3.16 ± 0.11	3.07 ± 0.12	0.616

[1] Low and [2] High inclusion levels of synthetic or encapsulated lysine.
[3] Mean fibre diameter
[4] Coefficient of variation of Fibre diameter
[5] Standard Deviation of fibre diameter

Table 3.7 shows the comparison between treatments of unprotected and protected lysine. Staple length differed significantly between these treatments. The staple length of the unprotected treatments was higher compared to protected treatments. In contrast, crimp showed a tendency ($P \leq 0.10$) to be larger for the protected treatments compared to the unprotected treatments. These findings may be the single most important factor influencing the shorter staple length as observed in this study. Staple length is a function of the frequency of

crimp (Duncan and Heitz, 1974). No other wool parameters in Table 3.7 showed any significant differences between protected and unprotected treatments.

Table 3.7 Wool parameters of Merino sheep receiving diets with unprotected lysine and protected lysine.

Wool parameters	Unprotected[1]	Protected[2]	*P*
C of V[4] (%)	19.17 ± 0.59	19.18 ± 0.48	0.984
SDFD[5]	3.81 ± 0.16	3.74 ± 0.108	0.704
Comfort factor (%)	98.31 ± 0.63	98.93 ± 0.25	0.357
Yield (%)	74.10 ± 1.00	73.51 ± 0.89	0.634
Crimp	9.75 ± 0.29	10.5 ± 0.29	0.071
Staple length	60.85 ± 0.89	55.2 ± 1.18	0.001
Strength	43.7 ± 2.48	40.45 ± 3.05	0.410
Tip	48.1 ± 9.87	36.2 ± 7.84	0.343
Middle	51.4 ± 9.99	63.3 ± 7.73	0.346
Greasy fleece weight	3.15 ± 0.11	3.08 ± 0.12	0.658

[1] Unprotected synthetic lysine and [2] Encapsulated commercial lysine

[3] Mean fibre diameter

[4] Coefficient of variation of fibre diameter

[5] Standard deviation of fibre diameter

In Table 3.8, the blood plasma lysine levels at two months into the trial were adjusted by including the blood plasma lysine levels of each animal before the trial started as a covariate. A tendency to differ ($P \leq 0.10$) was observed between the LLU treatment and the HLU and HLP treatments. The LLP treatment did not differ significantly from any of the other treatments. The LLU treatment had the lowest value of lysine in the blood after two months and the HLP treatment had the highest value. Figure 3.2 can be used as a visual aid for the differences seen in Table 3.8. These tendencies are in accordance with what was expected. In a study done with beef cattle, where rumen protected lysine was supplemented in the diets, plasma lysine levels were significantly higher compared to a controlled diet with no supplementation (Xue *et al.*, 2011). In contrast to the latter study and in support to the results of the current study, Awawdeh (2016) reported similar blood plasma levels of both methionine and lysine when the dietary level of these rumen protected AA were increased.

Table 3.9 shows blood lysine values of dietary high and low lysine treatments, respectively. Significant differences ($P \leq 0.05$) were observed between the HL and LL treatments after the animals had received different diets for two months. These results are in accordance with the work done by Xue *et al.* (2011) where the increased amount of supplementation of an amino acid has resulted in higher blood plasma levels of the specific amino acid. In contrast to these results Table 3.9 indicates no differences between Unprotected and Protected treatments after the animals had received the different diets for two months. The non-significant difference is however in contrast with research where processing of an amino

acid to produce a product that is resistant to rumen degradation lead to increased blood plasma levels (Awawdeh, 2016).

Table 3.8 Blood parameters of Merino sheep of four different diets with either high or low levels of encapsulated and unprotected lysine (µmol/L).

Blood parameters	**LLU[1]**	**LLP[2]**	**HLU[3]**	**HLP[4]**	***P***
2 Month[6] plasma	183.98[b] ± 14.65	223.65[ab] ± 14.40	240.28[a] ± 19.09	248.56[a] ± 22.94	0.098

[ab] Mean within rows with different superscripts differ ($P \leq 0.05$)

[1] Low level unprotected lysine, [2] Low level encapsulated lysine, [3] High levels unprotected lysine and [4] High levels encapsulated lysine

[5] Blood samples collected after 2 months of the trial period

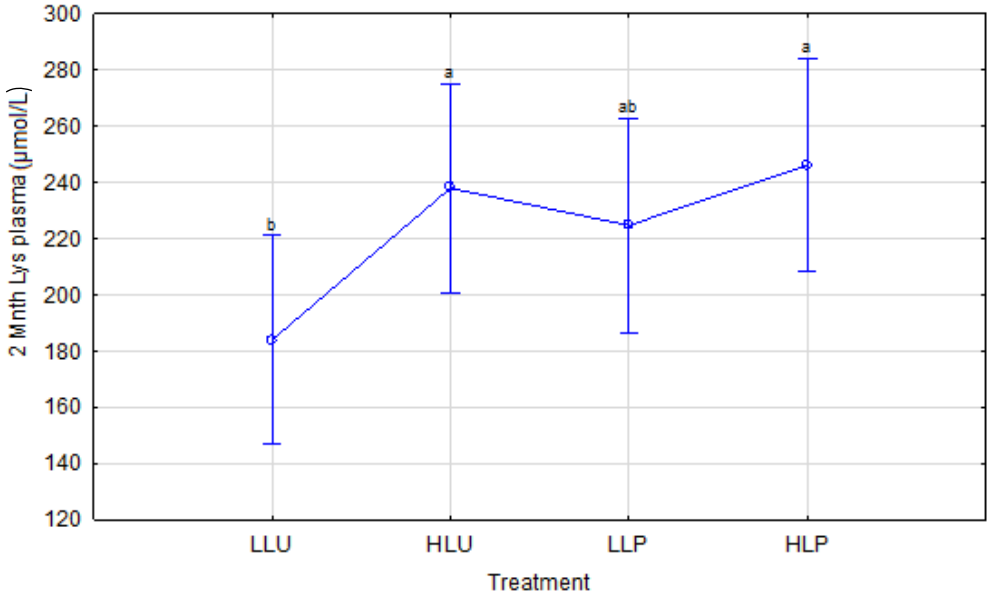

Figure 3.2 Graphical representation of blood plasma lysine levels of four different diets with either high or low inclusion levels of lysine or protected and unprotected lysine.

Table 3.9 Blood parameters of Merino sheep receiving diets with low and high inclusion levels of synthetic lysine compared to diets with protected and unprotected lysine (µmol/L).

Blood parameters	**LL[1]**	**HL[2]**	***P***
2 Month[5] plasma	203.94 ± 10.95	242.51 ± 14.57	0.048
	Unprotected[3]	**Protected[4]**	***P***
2 Month[5] plasma	210.90 ± 13.45	235.55 ± 13.50	0.20

[1] Low and [2] High inclusion levels of synthetic or encapsulated lysine. [3]Unprotected synthetic lysine and [4] Encapsulated commercial lysine. [5] Blood samples collected after 2 months of the trial period

3.5 Conclusion

Results of this study showed that the supplementation of by-pass lysine in the diet of Merino rams had a significant effect on DMI, but the response depended on the level of supplementation. When low levels of lysine were supplemented, by-pass lysine decreased DMI, but the opposite was observed when high levels were supplemented. Little response was seen when the wool parameters were analysed for all four treatment diets. The blood plasma levels measured were only significantly affected by the higher levels of supplementation in the diets and not by degradability.

The absence of response seen in the wool production parameters warrants future studies of longer duration to ensure more wool growth variation between the different treatments. Due to the decision to test the influence of lysine on wool production parameters, it was concluded that for future studies it would be advised to further research the responses of rumen protected sulphur containing AA on wool production parameters.

3.6 References

Allden, W., 1979. Feed intake, diet composition and wool growth. In P. R. JL Black (Ed.), Physiological and environmental limitations to wool growth (pp. 61–78).

AOAC, 2005. Official methods of analysis (18th Ed.). Association of Official Analytical Chemists, Inc., Washington, DC, USA.

Awawdeh, M.S., 2016. Rumen-protected methionine and lysine: effects on milk production and plasma amino acids of dairy cows with reference to metabolisable protein status. Journal of Dairy Research, 83, 151–155.

Bidinost, F., Roldan, D.L., Dodero, A.M., Cano, E.M., Taddeo, H.R., Mueller, J.P., and Poli, M.A., 2008. Wool quantitative trait loci in Merino sheep. Small Ruminant Research, 74, 113–118.

Botha, A.F. and Hunter, L., 2010. The measurement of wool fibre properties and their effect on worsted processing performance and product quality. Part 1: The objective measurement of wool fibre properties. Textile Progress, 42(4), 227–339.

Brusch, H., 2012. Histones and other nuclear proteins. Elsevier.

Cape wools SA, 2019. *Wool production reports*. < www.capewools.co.za >

De Barbieri, I., Hegarty, R.S., Li, L. and Oddy, V.H., 2015. Association of wool growth with gut metabolism and anatomy in sheep. Livestock Science, 173, 38–47.

Dlodlo, N., Botha, A.F. and Metelerkamp, R.G.M., 2009. A distributed knowledge-based system for the optimum utilisation of South African wool. Suid-Afrikaanse tydskrif vir natuurwetenskap en tegnologie, 28(3), 187–204.

Duncan, W.L. and Heitz, R.G., 1974. Measuring length and velocity of single staple fibre within an airflow. (Cited by Meissnet *et al., 1989)*

Fahmy, M.H., Boucher, J.M., Poste, L.M., Gregoire, R., Butlers, G. and Comeau, J.E., 1992. Feed efficiency, carcass characteristics, and sensory quality of lambs, with or without prolific ancestry, fed diets with different protein supplements. J. Anim. Sci., 70, 1365–1374.

Friend, M.A. and Robards, G.E., 2006. Wool production and quality of three strains of Merino in a semi-arid environment under different grazing strategies. Austr. J. Experimental Agric. (46), 37– 44.

Galbraith, H., 2000. Protein and sulphur amino acid nutrition of hair fibre-producing Angora and Cashmere goats. Livestock Production Science, 64, 81–93.

Goering, H.K. and Van Soest, P.J., 1970. Forage fibre analyses (Apparatus, reagents, procedures and some application). In USDA Ed: Agricultural handbook no. 379.

Holtrop, S., Abeling, N.G.G. and Gennip, A.H., 2002. Recommendations to improve the quality of diagnostic quantitative analysis of amino acids in plasma and urine. Standard operating procedure, Dept. of Clinical Chemistry, AMC, Amsterdam, Netherlands.

Hong, P., Wheat, T. and Diehl, D., 2009. Analysis of Physiological Amino Acids with the MassTrak Amino Acid Analysis Solution. Waters Corp 2009, (Application Note: 720002903EN).

Huisman, A.E. and Brown, D.J., 2008. Genetic parameters for body weight, wool and disease resistance and reproduction traits in merino sheep. Genetic relationships between body weight traits and other traits. Australian Journal of Experimental Agriculture, 48, 1186–1193.

Hunter, L., Smuts, S. and Gee, E., 1985. The role of sheep breed in determining the relationship between wool fibre properties and worsted yarn and fabric properties. Proceedings of Seventh International Wool Textile Research Conference, 11–25.

IWTO, 1998. Specification IWTO-47–98. Measurement of the mean and distribution of fibre diameter using an optical fibre diameter analyser (OFDA).

Kamalak, A., Canbolat, O., Gurbuz, Y. and Ozay, O., 2005. Protected Protein and Amino Acids in Ruminant Nutrition. Journal of Science and Engineering, 8(2), 84–88.

Kaufmann, W. and Lupping, W., 1982. Protected proteins and protected amino acids for ruminants. In ED: E.L. Muller, I.H. Pike, and A.J.H. Vanes, Protein Contribution of Feedstuffs for Ruminants (UK).

Khan, M.J., Abbas, A., Ayaz, M., Naeem, M., Akhter, M.S. and Soomro, M.H., 2012. Factors affecting wool quality and quantity in sheep. Afr. J. Biotechnol., 11(73), 13761–13766.

Kincaid, R.L. and Cronrath, J.D., 1993. Effects of added dietary fat and amino acids on performance of lactating cows. J. of Dairy Sci. 76(6), 1601–1606.

Kuffner, H. and Popescu, C., 2012. Wool fibres. Ed: R. M. Koslowski.

Kung Jr.L. and Rode L.M., 1996. Amino acid metabolism in ruminants. Animal Feed Science Technology, 59, 167–172.

Li, L., Oddy, V.H. and Nolan, J.V., 2008. Whole-body protein metabolism and energy expenditure in sheep selected for divergent wool production when fed above or below maintenance. Australian Journal of Experimental Agriculture, 48, 657–665.

Malik, R.C., Razzaque, M.A., Abbas, S., Al-Khozam, N. and Sahni, S., 1996. Feedlot growth and efficiency of three-way cross lambs as affected by genotype, age and diet. Proc. Aust. Soc. Anim. Prod, 21(251–254).

Mcgregor, B.A., De Graaf, S.P. and Hatcher, S., 2016. On-farm factors affecting physical quality of Merino wool. 1. Nutrition, reproduction, health and management. Small Ruminant Research, 137, 138–150.

National Research Council, 2007. Committee on Nutrient Requirements of Small Ruminants. Nutrient requirements of small ruminants : sheep, goats, cervids, and New World camelids. Washington, DC: National Academies Press, USA.

Ocak, S., Ogun, S. and Yilmaz, O., 2016. Dorper sheep utilizing feed resources efficiently: a Mediterranean case study. R. Bras. Zootec. 45(8), 489–498.

Patton, R. A., 2010. Effect of rumen-protected methionine on feed intake, milk production, true milk protein concentration, and true milk protein yield, and the factors that influence these effects: A meta-analysis. Journal of Dairy Science, 93, 2105–2118.

Ramada, W.A., El-Harairy, M. A., Khalil, W. A. and Youssef, A. H., 2017. Impact of Adding Rumen Protected Lysine or / and Methionine on Some Wool Characteristics in Barki Sheep. J. Anim. and Poultry Pod, 8(7), 173–177.

Rogers, G.R., 1964. Isolation and properties of inner sheath cells of hair follicles. Experimental Cell Research, 33(64), 81032- 81036

Safari, E., Fogarty, N.M. and Gilmour, A.R., 2005. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science, 92, 271–289.

Savary, I.C., Hoskin, S.O., Dennison, N. and Lobley, G.E., 2001. Lysine metabolism across the hindquarters of sheep; effect of intake on transfers from plasma and red blood cells. British Journal of Nutrition, 85, 565–573.

Statistica data analysis software system. 2017. Retrieved from http://statistica.io.

Vizard, A. L., and Williams, S. H. (1993). A model to estimate the economic value of using individual fleece fibre diameter measurements to class wool. Agricultural Systems, *41*(4), 475–486.

Wang, B., Ma, T., Deng, K.D., Jiang, C.G. and Diao, Q.Y., 2016. Effect of urea supplementation on performance and safety in diets of Dorper crossbred sheep. Journal of Animal Physiology and Animal Nutrition, 100(5), 902–910.

Xue, F., Zhou, Z., Ren, L. and Meng, Q., 2011. Influence of rumen-protected lysine supplementation on growth performance and plasma amino acid concentrations in growing cattle offered the maize stalk silage/maize grain-based diet. Animal Feed Science and Technology, 169, 61–67.

Chapter 4:
The *in vitro* determination of ruminal and intestinal digestibility of protected lysine

4.1. Abstract

The purpose of this study was to determine the in vitro disappearance of a commercial lysine product in the rumen and the lower digestive tract by means of a modified Ross Assay. The extent of lysine degradation was determined by an in vitro degradability method. The trial was done as a randomized block design with two types of commercial lysine products (rumen degradable and rumen protected) done in six replications using three ruminally cannulated donor cows in two separate runs. The Ross Assay was done in two phases where the first phase simulated ruminal degradation and the second phase abomasal and small intestinal digestion. Results from this study have indicated that there were no significant differences in ruminal degradation between the rumen degradable and rumen protected lysine products. Similarly, no significant differences in intestinal digestion have been observed between the two products. It was concluded that the encapsulation of the specific lysine product did not decrease rumen degradation or increase subsequent intestinal digestion.

4.2. Introduction

Amino acids (AA) are the building blocks of all proteins required for the optimal growth, production and maintenance of animals. However, when it is fed to ruminants, such as sheep, it is difficult to predict the quality and quantity of the AA that will be absorbed by the animal. These uncertainties are due to the microbial population in the rumen. The ingested AA are subjected to microbial degradation and the AA that are absorbed from the small intestine originate from microbial protein (MP) or dietary AA that escaped rumen degradation (Kung and Rode, 1996). Although MP alone is likely to meet the maintenance requirements of an animal (Kamalak *et al.,* 2005), it is insufficient to meet the optimal production demands of high producing ruminants (Kaufmann and Lupping, 1982).

Unfortunately, it is difficult to predict the nutritional availability of AA in the lower digestive tract (LDT) of ruminants, because of the large quantities of AA that are subject to microbial degradation in the rumen (Ohsumi *et al.,* 1994). Much research has been done in the development and treatment of protein sources to protect proteins from rumen degradation, increase the supply of proteins for production and to reduce nitrogen losses in the form of urea via the urine (Annison, 1981). Other research showed that by increasing the availability of AA in

the LDT increased animal production, including wool growth (Kung and Rode, 1996). A variety of methods and techniques have been developed to increase the supply of AA to the small intestine. Decreased AA retention time in the rumen also results in lower degradation values (Kamalak *et al.*, 2005).

The most common method to date is to feed a diet that is high in by-pass proteins (Kung and Rode, 1996). Other methods may include treating the protein or AA source with heat or chemicals or identifying proteins and AA's that are naturally protected (Kaufmann and Lupping, 1982). These methods can increase the availability of AA in the small intestine without increasing nitrogen losses (Kaufmann and Lupping, 1982). By chemical or physical alterations, these AA may become protected from rumen degradation. These methods include heat treatment, were the Maillard reaction caused by a heating proses enables the protein to be more resistant to the enzymatic hydrolysis of rumen microbes (Belits *et al.*, 2005). Formaldehyde treatment may be applied where cross-links between the amino groups of protein are formed and thus reduce the susceptibility to microbial degradation in the rumen (Czerkawski, 1986). Both these methods may increase the percentage of proteins and AA that reach the small intestine and thus increase the availability of nutrients to the animal for absorption and production. However, excessive processing might have a negative effect on the digestion of the diet (Manterola *et al.*, 2001).

Currently, various attempts have been made to develop and produce AA that resist digestion in the rumen. These methods include structural manipulation to produce AA analogues and AA coated with resistant materials (Kamalak *et al.*, 2005). The encapsulated AA consists of a core containing the specific AA and a pH sensitive coating that is synthetically produced (Kung and Rode, 1996). The capsule can thus resist degradation in the higher pH of the rumen but it will be degraded at the lower pH of the LDT (Ohsumi *et al.*, 1994). It has been shown that encapsulation can result in an increase in the availability of a specific AA in the small intestine of the ruminant (Kung and Rode, 1996), thereby creating by-pass AA.

The utilization of nutrients post-rumen has advantages for the animal. For example, energy losses that are related to fermentation in the rumen may be reduced and the loss of protein sources when dietary proteins are transformed to MP may also be reduced. It must be taken into consideration that the protein digestive process in a ruminant may not be as efficient as in a monogastric animal when the site of absorption of nutrients are changed, from the rumen to the small intestine (Kamalak *et al.*, 2005). Another advantage of protecting AA against ruminal degradation is that the percentage of free AA to be used for production are higher in the small intestine. When supplementing AA in the diet of ruminant animals through the use of by-pass AA, consideration to the limiting AA associated with the particular physiological status or production of the animal will be required (Chalupa, 1975).

Despite these advantages associated with the use of rumen undegradable proteins or by-pass AA, there is still some considerable lack of responses seen in research. This can be due to different factors (Schingoethe, 1996):

- The first may be that the higher degree of by-pass of proteins/AA through the rumen could result in a negative effect on MP synthesis.
- The second factor could be that digestion of these by-pass protein/AA sources is poor in the small intestine and they pass through the entire digestive tract.
- A third factor could be that the proteins/AA that by-pass to the small intestine are inefficient to the limiting AA requirement for production.

The purpose of this study was, therefore, to determine the degradation characteristics of a commercial coat-protected AA (lysine) in the rumen and the consequent digestion in the lower digestive tract by means of the Ross Assay (Ross *et al.*, 2008).

4.3. Materials and Methods

All research work for this trial was conducted in agreement with the Stellenbosch University Ethics Committee (Reference AUC-2018-6493).

4.3.1. General

In this trial, the extent of lysine degradation and intestinal digestibility were determined by a three-step *in vitro* method, referred to in this chapter as the Ross Assay (Ross *et al.,* 2008). The trial was done as a randomized block design with two types of commercial lysine (rumen degradable and rumen protected) with six replications, using rumen fluid of three cows in two separate runs.

4.3.2. Preparations

The two types of commercial lysine were received from a commercial animal feed supplier. Prior to the start of the Ross Assay, all the required solutions were prepared, namely:

- positive control (freeze-dried blood)
- negative control (burnt blood meal)
- neutral detergent residue from corn silage (CSND).

The positive control was prepared by freeze drying fresh poultry blood. The poultry blood was processed less than 12 hours from collection. The negative control was prepared from the freeze-dried poultry blood that was heated in an oven at 130 ºC for 12 hours. This process was repeated until a pepsin digestibility of less than 20 % was achieved.

The CSND was prepared as seen in Table 4.1 by washing 120 g of dried and ground corn silage with boiling Neutral Detergent (ND) solution for seventy-five minutes. The residue was

then rinsed three times with boiling water before being dried overnight in a force draught oven (105 ºC). The CSND was then incubated (18 hours at 39 ºC) in ammonium sulphate (1 M) to remove the sodium lauryl sulphate in the ND solution that would inhibit bacterial growth. After incubation, the CSND residue was filtered out and washed three times with boiling water and dried in a force draught oven overnight. The CSND was then milled through a 2 mm screen and stored in an airtight honey jar until used in the Ross Assay. A sample of 0.5 g of each commercial product was weighed out and transferred to labelled 250 ml Erlenmeyer flasks the day before the assay.

Table 4.1 Composition of the solution used to isolate CSND.

Reagent	**Quantity**
1L ND[1] Solution:	
EDTA disodium salt ($C_{10}H_{14}N_2Na_2O_2.2H_2O$)	18.61 g
Sodium tetra borate ($Na_2B_4O_7.10H_2O$	6.81 g
Disodium hydrogen orthophosphate (Na_2HPO_4)	4.56 g
Sodium lauryl sulphate ($CH_3(CH_2)_{10}CH_2OSO_3Na$)	30 g
Distilled water (dH_2O)	1000 mL
Triethylene glycol($C_4H_{10}O_2$)	10 mL
1L Ammonium sulphate solution (1M):	
Distilled water (dH_2O)	1000 mL
Ammonium sulphate $(NH_4)_2SO_4$	132.14 g

[1] Neutral Detergent solution (Goering and Van Soest, 1970)

4.3.3. Ross Assay

In this study the Ross Assay (Ross *et al.*, 2008) was used as a method to estimate the rumen degradation as well as the intestinal digestion of two products. The Assay was modified by keeping Phase 1 (rumen degradation) separate and combining Phase 2 and 3 (complete intestinal digestion) to suit our objectives for this study.

4.3.3.1. Rumen fluid collection

According to research by Mould *et al.* (2005) and Witzig *et al.* (2015) on the use of cow versus sheep rumen fluid for an *in vitro* study, the time of rumen fluid collection (pre- or post-feeding) has a much greater influence on results than the donor species. Thus, since rumen fluid of either specie can be used, it was decided to use cows as donors because of their availability on the University's Experimental Farm.

Rumen fluid was collected from two ruminally cannulated lactating Holstein dairy cows on the morning of the *in vitro* assay. Collection for this trial commenced at 07h30 in the morning after milking. All rumen fluid collections were done according to the rumen fluid extraction protocol of the University of Stellenbosch. Cows used in this trial were from the Welgevallen Experimental Farm's herd of the Stellenbosch University, South Africa. The cows had *ad libitum* access to a TMR that consisted of lucerne hay (310 g/kg DM) and wheat straw (18 g/kg DM), mixed with a commercial lactating cow concentrate (619 g/kg DM) and molasses meal (53 g/kg DM). Water was added to balance the moisture content of the TMR to 550 g/kg. The cows received the feed twice daily at 07h00 and 16h00.

To ensure a homogenous collection of rumen fluid, the fluid was collected from multiple areas in the rumen and filtered through two layers of cheesecloth before transportation to the laboratory. The fluid was transported in pre-warmed thermos flasks, filled to the brim to ensure an anaerobic environment of the content. Upon arrival at the laboratory, the content of each thermos flask was strained through four layers of cheesecloth into prewarmed Erlenmeyer flasks (39 ºC) after which each flask was gassed with a steady stream of CO_2 to ensure an anaerobic environment of the content until use.

4.3.3.2. Phase 1

Prior to the start of the *in vitro* analysis of the two commercial lysine products, all the *in vitro* solutions were prepared as described by Goering and Van Soest (1970) in Table 4.2. The solution was prepared by mixing a macro-mineral solution, buffer solution, micromineral solution, rezasurin solution and a reducing solution. The macro-mineral, buffer and micromineral solutions were kept at room temperature and the rezasurin solution was kept in a fridge at 4 ^{0}C before it was added to the incubation medium. The incubation medium was mixed one day before the *in vitro* analysis and placed in the incubation room to reach the incubation temperature of 39 ºC. The reducing solution was prepared by firstly mixing certain chemicals in separate flasks, A and B, and subsequently gently mixing these flasks. The content of flask B was mixed with the content of flask A and placed in the incubation room to reach the incubation temperature of 39 ºC. Prior to the onset of incubation, the reducing solution was mixed with the incubation medium.

Using a surgical syringe, 40 mL of the *in vitro* solution and a magnetic stirrer were added to each 250 mL Erlenmeyer flask that contained 0.5 g samples of either the positive control, negative control, CSND, unprotected lysine or protected lysine. To two of the flasks with CSND, an amount of 50 mL *in vitro* solution was added, this served as a correction factor for microbial contamination of the samples. To each of the remaining flasks that contained 40 ml *in vitro* solution, a volume of 10 mL of strained rumen fluid was added with a surgical syringe. In order to keep the contents anaerobic all the flasks were subsequently gassed with a steady stream of

CO_2 where after a rubber stopper fitted with a one-way valve was inserted into each flask. All the flasks were transferred to the incubation room and placed on magnetic stirrer plates. The temperature of the incubation room was maintained at 39 ºC throughout the incubation period.

For this assay, the samples were incubated for 12 hours. At the end of the incubation period flasks were acidified by adding 2 mL of 3 M HCl and removed from the incubation room according to the schedule in Table 4.3. The flask contents were carefully transferred into Buchner funnels and filtered under vacuum through a pre-weighed 11 cm glass microfiber filter with a 1.5 µm pore size (Whatman 934-AH). The flasks were rinsed with 10 mL of distilled water and after filtration all the filters were dried at 100 ºC for 12 hours.

4.3.3.3. Phases 2 and 3

Two mL of a pepsin solution were added to all the remaining flasks that remained in the incubation room (as indicated in Table 4.3). This solution comprised of 0.013 M HCl and 0.6 g pepsin per 1 L solution. The samples were then further incubated for 1 hour at 39 ºC. After incubation, the solutions were neutralized to approximately pH 5 using 2 mL of 2 M NaOH. At the end of the 1-hour incubation period, 10 mL of 1.8 M potassium phosphate buffer, containing various enzymes and with a pH of 7.75, were added. This enzyme cocktail comprised of 24 U/mL of trypsin, 20 U/mL of Chymotrypsin, 50 U/mL of amylase, 4 U/mL of lipase and 1 g/L of bile. The remaining flasks were incubated for a further 24 hours at 39 ºC and acidified at the end of the incubation period with 2 ml of 3 M HCl solution to inhibit further enzyme activity. The content of each flask was carefully transferred into Buchner funnels and filtered under vacuum through a pre-weighed 11 cm glass microfiber filter with a 1.5 µm pore size (Whatman 934-AH). The flasks were rinsed with 10 mL of distilled water and after filtration all the filters were dried at 100 ºC for 12 hours.

Table 4.2 The composition of the *in vitro* solutions (Goering and Van Soest, 1970)

Reagent	Quantity
1L Macro-mineral solution	
Di-sodium hydrogen orthophosphate (Na_2HPO_4) (anhydrous)	5.7 g
Potassium dihydrogen orthophosphate (KH_2PO_4) (anhydrous)	5.2 g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.6 g
Distilled water (dH_2O)	1000 mL
1L Buffer solution:	
Ammonium bicarbonate (NH_4HCO_3)	4 g
Sodium bicarbonate ($NaHCO_3$)	35 g
Distilled water (dH_2O)	1000 mL
100 mL Micro-minerals	
Calcium chloride dihydrate ($CaCl_2.2H_2O$)	13.2 g
Manganese chloride tetrahydrate ($MnCl_2.4H_2O$)	10 g
Cobalt (II) chloride hexahydrate ($CoCl_2.6H_2O$)	1 g
Ferric chloride hexahydrate ($FeCl_3.6H_2O$)	8 g
2.4L Incubation medium (60 samples):	
Distilled water (dH_2O)	1200 mL
Tryptose	6 g
Micro mineral solution	300 µL
Macro mineral solution	600 mL
Buffer solution	600 mL
Rezasurin	3 mL
120 mL Reducing solution (60 samples):	
Flask A:	
Cysteine Hydrochloride ($C_3H_7NO_2HCl$)	0.750 g
Potassium hydroxide (KOH) pellets	30
Distilled water (dH_2O)	60 mL
Flask B:	
Sodium sulphide monohydrate (NaS)	0.750 g
Distilled water (dH_2O)	60 mL
***In vitro* Solution**:	
Incubation medium	2.4 L
Reducing solution	120 mL

Table 4.3 Graphical explanation of sample removal after each phase of Ross Assay

Samples	Containers removed
Phase 1	CSND[1], product[4]
Phase 2 & 3	Positive control[2], negative control[3], product[4]

[1] all containers containing CSND were removed.
[2] containers containing positive control (freeze-dried blood) were removed.
[3] containers containing negative control (baked blood) were removed.
[4] containers containing product were removed per treatment.

4.3.4. Amino acid analysis

Amino acid analyses were conducted as described by Salazar *et al.* (2012). The entire filter paper was transferred to a glass tube and 10 ml of 6M HCl containing 1 g of phenol and 100 mg/L of Norleucine were added. The tubes were placed in hot water for 10 minutes then capped and transferred to an oven at 110 °C. Hydrolysis proceeded for 21 hours, where after the tubes were allowed to cool and the hydrolysate filtered into a 15 ml falcon tube. The samples were diluted 5 times by mixing 200 µl of sample, 200 µl of Norvaline internal standard, 200 µl of borate buffer, 200 µl of 6 M NaOH and 200 µl of water. Derivatization (using AccQ-tag Ultra amino acid kit from Waters) of the samples was performed by adding 70 µl of borate buffer, 10 µl of sample-standard and 20 µl of AQC derivatizing agent into a 200 µl glass insert. After capping the vials were vortexed and then derivatized at 55 °C for 10 minutes. Thereafter the vials were transferred to the autosampler for AA analysis.

4.3.5. Statistical analyses

Since the trial was a randomized block design, a main effects ANOVA (treatment and block) was used to compare the response variables versus the two factors, namely protected lysine (by-pass lysine) and unprotected lysine (rumen degradable lysine) using STATISTICA, version 13 (TIBCO Software Inc., 2017). When ordinal response variables were compared versus a nominal input variable, non-parametric ANOVA methods were used. For the repeated measures in a completely randomized design, the Wilcoxon matched-pairs test was used. A *P*-value of $P \leq 0.05$ represent statistical significance in the hypothesis testing and a *P*-value of $P \leq 0.10$ represent a tendency.

4.4. Results and Discussion

In vitro rumen degradation values of both protected and rumen degradable lysine were determined after the completion of Phase 1 of the Ross Assay. Results are presented in Table 4.4. When the protected lysine was compared with rumen degradable lysine, no significant differences were observed. As seen from the results more than 95% of the lysine added at the beginning of the Assay were degraded in the first phase of the Assay which simulated the rumen environment. The non-significant difference between the rumen degradability (RD) of protected lysine and rumen degradable lysine is an indication that the encapsulation used to produce the protected lysine did not decrease the degradation of the product after 12 hours of incubation.

The high level of rumen degradation seen in Table 4.4 may be due to the 12-hour incubation time of Phase 1 of the Ross Assay. However, the passage rate of undigested feed from the rumen is the reciprocal of mean retention time (MRT), kp = 1/MRT (Poppi *et al.*, 1981; Cochran *et al.*, 1987). Therefore, an incubation time of 12 hours corresponds to a passage rate of 0.08 that is generally accepted for lactating dairy cows with a high DMI. According to the suppliers of the protected lysine product, the expected MRT of the protected lysine product is no more than 8 hours. However, this is probably not possible, as the MRT of the liquid phase in the rumen is 8 hours (Hartnell and Satter, 1979) and it is highly unlikely that any particle matter would have a similar retention time. In contrast, a 3-step approach was developed by Calsamiglia and Stern (1995) to quantify the intestinal digestibility of proteins passing through the rumen. This was an *in situ* incubation process were the N-residues were collected after 16 hours of incubation.

The suppliers of the product have also indicated that the specific gravity (SG) of the protected lysine product is close to 1.2 and it had a mean volume of 0.033 cm^3. Welch and Smith (1978) indicated in their study that the most rapid passage rate for particles of 0.03 cm^3 in size and an SG of 1.2 was between kp = 0.028 and kp = 0.042. These findings were also confirmed by Des Bordes and Welch (1984) were they studied the influence of specific gravity on rumination and passage of indigestible particles. In another study, Kaske and Engelhardt (1990) evaluated a series of inert plastic particles of different sizes and specific gravities and found that the MRT of 1 mm particles with an SG of 1.22 was 35.5 hours, which is equivalent to a kp of 0.028.

In the current study we also observed that when the protected lysine product was removed after Phase 1 the encapsulation of the product was soft and did not retain its structural integrity. This could have caused some of the lysine in the core of the encapsulated product to leach out and thus be degraded in the ruminal phase of the Ross Assay. When considering the movement of digesta in the rumen as described by Cuthbertson and Balch (1959), it could

cause the encapsulation to lose its function and subsequently cause the content of the product to be available in the rumen, due to the softening of the encapsulation in the rumen fluid.

Table 4.4 Percentage (%) of commercial lysine product degraded in the ruminal phase of the Ross Assay

Lysine product (%)	RD [1]	SEM	*P*
Protected [2]	96.414	0.541	0.311
Unprotected [3]	97.679	0.784	

[1] Rumen degraded percentage.
[2] Protected lysine (By-pass lysine).
[3] Unprotected lysine (Rumen degradable lysine).

The intestinal digestion of both the protected and degradable lysine products are summarized in Table 4.5. Determination of the intestinal digestion was done after Phase 3 of the Ross Assay due to the objective that the total digestion in the LDT should be measured. When the two products were compared, no significant differences were again observed. For both the lysine products the intestinal digestion of the sample after Phase 3 was more than 95%. The intestinal digestion is an indication of both the abomasal digestion and the small intestine enzymatic digestion. The absence of significant differences between products in terms of intestinal digestibility (ID) is a further confirmation that the encapsulation material and process used to produce the protected lysine did not increase the availability of lysine in the lower pH environment of the LDT as more than 95% of the product had already been degraded after Phase 1.

The lack of difference between the protected and degradable lysine products is in contrast with research done by Broderick *et al.* (1991) who showed that processing of amino acids through either heat treatment, chemical treatment (formaldehyde) or structural alteration should lead to reduced ruminal degradation and subsequently increased intestinal digestion. Research done by Socha *et al.* (2005), also confirmed that feeding rumen protected amino acids should decrease rumen degradation and increase intestinal digestion and absorption of the encapsulated product. It was also stated in further studies by Socha *et al.* (2005), that the simulation of the ruminal and intestinal environments should have the same response as mentioned above.

Calsamiglia and Stern (1995) stated that the fraction of proteins/AA that escapes the rumen is a function of both the degradation rate and the passage rate of the product studied. Thus, due to a large amount of rumen degradation of the product already in the rumen, little post ruminal response was observed.

Table 4.5 Percentage (%) of commercial lysine product digested in the intestinal phase of the Ross Assay

Lysine product (%)	ID [1]	SEM	*P*
Protected [2]	97.076	0.517	0.164
Unprotected [3]	96.081	0.382	

[1] Intestinal digested percentage.
[2] Protected lysine product (By-pass lysine).
[3] Unprotected lysine product (Rumen degradable lysine).

4.5. Conclusion

The results of the current study showed that the specific encapsulation material used in the production of the rumen protected lysine product was not successful in resisting microbial degradation of the lysine. It did also not increase the subsequent intestinal availability of lysine in the LDT for absorption.

The lack of response seen in this study indicates that future studies should be developed to produce an encapsulation technique that can withstand rumen degradation for up to at least 12 hours without reducing structural integrity. Future researchers should also investigate the response of encapsulated products in an *in sacco* trial with different incubation times to estimate the level of degradation of the product.

4.6. References

Annison, E. F., 1981. The role of protein which escapes ruminal degradation. *(Recent Advantages in Animal Nutrition in Australia)* (Unit; D. J. Farrell, Ed.).

Belits, H.D., Grosch, W. and Schieberle, P., 2005. Reaction involved in food chemistry. *In: Food Chemistry.* (3rd ed.; Springer Berlin, Ed.). R

Broderick, G. A., Wallace, R. J.,and Ørskov, E. R., 1991. Control of Rate and Extent of Protein Degradation. In K. T., Tsuda, Y., Sasaki, R. (Ed.), *Physiological Aspects of Digestion and Metabolism in Ruminants* (CA, pp. 541–592).

Calsamiglia, S. and Stern, M. D., 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. *Journal of Animal Science*, *73*(5), 1459–1465.

Chalupa, W., 1975. Rumen bypass and protection of proteins and amino acids. *Journal of Dairy Science*, *58*(8), 1198–1218.

Cochran, R. C., Adams, D. C., Galyean, M. L. and Wallace, J. D., 1987. Examination of Methods for Estimating Rate of Passage in Grazing Steers. *Journal of Range Management*, *40*(2), 105–108.

Cuthbertson, D. P. and Balch, C. C., 1959. Rumen function: Structure of the ruminant stomach and the movement of its contents. *Proceedings of the Nutrition Society*, 97–102.

Czerkawski, J. W., 1986. An introduction to rumen studies (1st ed.).

Des Bordes, C. K. and Welch, J. G., 1984. Influence of Specific Gravity on Rumination and Passage of Indigestible Particles. *Journal of Animal Science*, *59*(2), 470–475.

Goering, H. K. and Van Soest, P. J., 1970. Forage fibre analyses (Apparatus, reagents, procedures and some application). In USDA (Ed.), *Agricultural handbook no. 379*.

Hartnell, G. F. and Satter, L. D., 1979. Determination of Rumen Fill, Retention Time and Ruminal Turnover Rates of Ingesta at Different Stages of Lactation in Dairy Cows. *Journal of Animal Science*, *48*(2), 381–392.

Kamalak, A., Canbolat, O., Gurbuz, Y. and Ozay, O., 2005. Protected Protein and Amino Acids in Ruminant Nutrition. *Journal of Science and Engineering* , *8*(2), 84–88.

Kaske, M. and Engelhardt, W. V., 1990. The effect of size and density on mean retention time of particles in the gastrointestinal tract of sheep. *Brirish Journal of Nutrition*, *63*, 457–465.

Kaufmann, W. and Lupping, W., 1982. Protected proteins and protected amino acids for ruminants. In E.L. Muller, I.H. Pike, and A.J.H. Vanes (Eds.), *Protein Contribution of Feedstuffs for Ruminants* (UK).

Kung Jr. L. and Rode L.M., 1996. Amino acid metabolism in ruminants. *Animal Feed Science Technology*, *59*, 167–172.

Manterola, H. B., Cerda, D. A. and Mira, J. J., 2001. Protein degradability of soybean meal coated with different lipid substances and its effects on ruminal parameters when included in steer rations.

Mould, F. L., Kliem, K. E., Morgan, R. and Mauricio, R. M., 2005. In vitro microbial inoculum: A

review of its function and properties. *Animal Feed Science and Technology*, *123*, 31–50.

Ohsumi, T., Sato, H., Yoshihara, Y. and Ikeda, S., 1994. Selection and Breeding of Lysine-accumulating Saccharomyces cerevisiae as a Stable Source of Lysine in the Rumen. *Biosci. Biotechnol. Biochem.*, *58*, 1302–1305.

Poppi, D. P., Minson, D. J. and Ternouth, J. H., 1981. Studies of Cattle and Sheep Eating Leaf and Stem Fractions of Grasses. III The Retention Time in the Rumen of Large Feed Particles. *Aust. J. Agric. Res*, *32*, 123–137.

Ross, D. A., Van Amburgh, M. E. and Gutierrez-Botero, M., 2008. Developement of an in vitro digestability assay for ruminant feeds. *Cornell Nutrition Conference for Feed Manufacturers*, (1963), 190–220. Ithaca, NY, Cornell University: Syracuse, NY.

Salazar, C., Armenta, J. M., Cortes, D. F. and Shuleav, V., 2012. Combination of an AccQ·Tag-ultra performance liquid chromatographic method with tandem mass spectrometry for the analysis of amino acids. *Methods In Molecular Biology (Clifton, N.J.)*, Vol. 828, pp. 13–23.

Schingoethe, D. J., 1996. Balancing the amino acid needs of the dairy cow. *Animal Feed Science Technology*, *60*, 153–160.

Socha, M. T., Putnam, D. E., Garthwaite, B. D., Whitehouse, N. L., Kierstead, N. A., Schwab, C. G. and Robert, J. C., 2005. Improving intestinal amino acid Supply of pre-and postpartum dairy cows with rumen-protected methionine and lysine. *Journal of Dairy Science*, *88*, 1113–1126.

Welch, J. G. and Smith, A. M., 1978. Particle sizes passed from rumen. *Journal of Animal Science*, *46*(1), 309–312.

Witzig, M., Boguhn, J., Zeder, M., Seifert, J. and Rodehutscord, M., 2015. Effect of donor animal species and their feeding on the composition of the microbial community establishing in a rumen simulation. *Journal of Applied Microbiology*, *119*(1), 33–46.

Chapter 5:
General conclusion and recommendations

5.1. Conclusion

The relatively high market price of R183,84 per kilogram of wool (top lines) and the dynamic versatility of wool encourages research to improve the production of wool and optimizing the cost of wool production. Amino Acids (AA) are the building blocks of all proteins required for optimal wool production. However, the level of microbial protein (MP) production in the animal is often a limiting factor for optimal production. The requirement to formulate feeds with specific AA profiles, as well as lower digestive tract (LDT) availability, is imperative. This has led to recent renewed interest in the development of rumen protected AA.

Results of the *in vivo* study showed that the supplementation of by-pass lysine in the diet of Merino rams had a significant effect on DMI, but the response depended on the level of supplementation. Little response was seen when the wool parameters were analysed for all four dietary treatments. The objective regarding the effect of lysine type (degradable or by-pass) on wool production parameters was thus found to be unsuccessful. The blood plasma levels measured were only significantly affected by the higher levels of lysine supplementation in the diets and not by degradability. Thus, the objective regarding the absorption of dietary lysine in the LDT was only achieved by the level of dietary lysine and not by degradation characteristics.

No significant differences could be established between the ruminal degradation (RD) of the by-pass lysine and rumen degradable lysine products. Intestinal digestibility also showed no significance between the by-pass lysine and the rumen degradable lysine products. Thus the results of the *in vitro* study indicated that the specific encapsulation of lysine did not decrease the ruminal degradation of lysine. Neither did rumen protection increase the intestinal availability of protected lysine in the LDT. To conclude, the *in vitro* study showed the same response as seen in *in vivo* study were the by-pass lysine product did not increase production parameters of the sheep as it did not decrease the ruminal digestion of lysine (as seen in the *in vitro* study).

5.2. Recommendations and future studies

The absence of responses observed in the wool production parameters warrants future studies of longer duration to ensure more wool growth variation between the different treatments. Due to the decision to test the effect of lysine on wool production parameters it was concluded that for future studies it would be advisable to further investigate responses of

rumen protected sulphur containing AA (methionine and cysteine) on wool production parameters.

The lack of response seen in the *in vitro* study indicated that future encapsulation studies should be designed to develop encapsulation techniques that can withstand rumen degradation for a minimum of 12 to 16 hours of incubation without losing its structural integrity. Future studies should also investigate the response of the encapsulated product in an *in sacco* trial that estimates levels of degradation at different timed intervals.